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ASSOCIATION BETWEEN DIET AND TREATMENT RESULTS IN PATIENTS WITH RHEUMATOID ARTHRITIS AND SYSTEMIC LUPUS ERYTHEMATOSUS

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Association between diet and treatment results in patients with rheumatoid arthritis and systemic lupus erythematosus

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To my godfather Sorna Rajan

(1942-2009)

ABSTRACT

Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are chronic autoimmune inflammatory diseases. The causes of these diseases are unknown and evidence indicates that a combination of both genetic and environmental factors contribute to their etiology. Several studies have reported dietary benefits for patients with RA as well as SLE. Nevertheless, many of these studies have mainly focused on the dietary impact on the disease status, and the affect on treatment has not often been considered. The majority of patients with RA and SLE are dependent on treatment. Therefore, the dietary aspects in regards to treatment need to be explored further. Thus, the aim of this thesis was to examine the association between diet and treatment results in patients with RA and SLE.

This thesis included five papers (**Papers I-V**) and was based on data of participants from three registers; Epidemiological Investigation of Rheumatoid Arthritis (EIRA), SLE Vascular Impact Cohort (SLEVIC) and Swedish Mammography Cohort (SMC). All participants from EIRA, SLEVIC and SMC were asked to complete food frequency questionnaires (FFQ) regarding their dietary habits. Dietary data from completed FFQ were linked with clinical data obtained from either Swedish Rheumatology Quality register (SRQ) or medical records. The associations between diet and clinical outcomes were analyzed with logistic regression and prospective dietary changes were analyzed with mixed models and cluster analysis.

The main results of this thesis showed that 1) vitamin D and omega-3 fatty acids (FA) were associated with better response to treatment in RA patients, 2) beta-carotene (antioxidant), linoleic acid (omega-6 FA) and vitamin B6 were inversely associated with increased doses of glucocorticoids in SLE patients, 3) women who have been diagnosed with RA did not remarkably change their diet due to their disease, 4) omega 3 FA was inversely associated with non-inflammatory pain after anti-rheumatic treatment in RA patients and 5) riboflavin, phosphorus, selenium and thiamin were inversely associated with carotid plaque in SLE patients.

Results from this thesis presented several associations between specific dietary nutrients and clinical outcomes of RA and SLE, in particular concerning treatment results. In summary, diet may play a role in response to anti-rheumatic treatment in patients with RA and SLE.

LIST OF SCIENTIFIC PAPERS

This thesis is based on five scientific papers listed below and will be referred to as **Papers I-V** throughout the thesis.

I. **Lourdudoss C**, Wolk A, Nise L, Alfredsson L, van Vollenhoven RF.
Associations between dietary intake of vitamin D, omega-3 fatty acids, folate and EULAR response in patients with early rheumatoid arthritis.
Submitted.

II. **Lourdudoss C**, Hafström I, Frostegård J, van Vollenhoven RF.
The association between diet and glucocorticoid treatment in patients with SLE.
Lupus Sci Med, 2016. **3**(1): p. e000135.

III. **Lourdudoss C**, Arnaud L, Wolk A, van Vollenhoven RF, Di Giuseppe D.
Long-term dietary changes after RA diagnosis in Swedish women: data from a population based cohort.
Submitted.

IV. **Lourdudoss C**, Di Giuseppe D, Wolk A, Klareskog L, Alfredsson L, van Vollenhoven RF, Lampa J.
Dietary intake of polyunsaturated fatty acids and pain despite of inflammatory control among methotrexate treated early rheumatoid arthritis patients.
Submitted.

V. **Lourdudoss C**, Elkan AC, Hafström I, Jogestrand T, Gustafsson T, van Vollenhoven RF, Frostegård J.
Dietary micronutrient intake and atherosclerosis in systemic lupus erythematosus.
Lupus, 2016, pii: 0961203316655211.

RELATED PAPERS

Related papers listed below will not be discussed in this thesis.

VI. **Lourdudoss C**, van Vollenhoven RF.
Mycophenolate mofetil in the treatment of SLE and systemic vasculitis: experience at a single university center.
Lupus, 2014. **23**(3): p. 299-304.

VII. Levitsky A, Brismar K, Hafström I, Hambardzumyan K, **Lourdudoss C**, van Vollenhoven RF, Saevarsdottir S.
Obesity is a strong predictor of starkly worse clinical outcomes in early rheumatoid arthritis: Results from the SWEFOT trial.
Manuscript.

CONTENTS

LIST OF ABBREVIATIONS

ACPA	Anti-Citrullinated Protein Antibody
ACR	American College of Rheumatology
ANA	Anti-Nuclear Antibody
AZA	Azathioprine
BILAG	British Isles Lupus Activity Group
BMI	Body Mass Index
CD	Cluster of Differentiation
CI	Confidence Interval
CRP	C-Reactive Protein
CVD	Cardiovascular Diseases
CyA	Cyclosporine
CYC	Cyclophosphamide
DAS	Disease Activity Score
DMARD	Disease Modifying Anti-Rheumatic Drugs
dsDNA	Double Stranded Deoxyribonucleic Acid
E%	Energy Percent
EIRA	Epidemiological Investigation of Rheumatoid Arthritis
ESR	Erythrocyte Sedimentation Rate
EULAR	European League Against Rheumatism
FA	Fatty Acids
FFQ	Food Frequency Questionnaires
GC	Glucocorticoids
GA	Global Assessment
HAQ	Health Assessment Questionnaire
HCQ	Hydroxychloroquine
HLA	Human Leukocyte Antigen
Ig	Immunoglobin
IL	Interleukin
IMT	Intima Media Thickness
IQR	Interquartile Range

LDL	Low Density Lipoprotein
LN	Lupus Nephritis
MCP	Metacarpophalangeal (joint)
MHC	Major Histocompatibility Complex
MMF	Mycophenolate Mofetyl
MTP	Metatarsophalangeal (joint)
MTX	Methotrexate
NSAID	Non-Steroid Anti-Inflammatory Drug
OR	Odds Ratio
PASS	Patients Acceptance Symptom State
PIP	Proximal Interphalangeal (joint)
PLP	Pyridoxal Phosphate
PUFA	Polyunsaturated Fatty Acids
RA	Rheumatoid Arthritis
RDI	Recommended Daily Intake
RF	Rheumatoid Factor
SD	Standard Deviation
SJC	Swollen Joint Count
SLAM	Systemic Lupus Activity Measure
SLE	Systemic Lupus Erythematosus
SLEDAI	Systemic Lupus Erythematosus Disease Activity Index
SLEVIC	Systemic Lupus Erythematosus Vascular Impact Cohort
SLICC	Systemic Lupus International Collaboration Clinics
Sm	Smith
SMC	Swedish Mammography Cohort
SRQ	Swedish Rheumatology Quality (register)
SSZ	Sulfasalazine
TJC	Tender Joint Count
TNF	Tumor Necrosis Factor
TRP	Transient Receptor Potential (channel)
VAS	Visual Analogue Scale

1 INTRODUCTION

Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are chronic autoimmune inflammatory diseases. The causes of these diseases are unknown and evidence indicates that a combination of both genetic and environmental factors contribute to their etiology. The global prevalence of RA is 0.5-1.0% [1], and the global prevalence of SLE varies based on age, gender and ethnic groups and is estimated 3-200 per 100,000 people [2]. Compared to RA, the disease activity of SLE can vary more between flares and remissions. Both RA and SLE are found predominantly in women .Additional characteristics of the two diseases will be discussed further in the sections ahead.

The interest in the influence of dietary factors in RA and SLE has increased over the last decade, both among patients and researchers. Several studies have reported dietary benefits for patients with RA and SLE. However, many of these studies have mainly focused on the dietary impact on the disease status, while the impact on treatment has not always been considered. The majority of patients with RA and SLE are dependent on treatment(s), therefore the link between diet and treatment results needs to be explored further. Thus, the aim of this thesis is to examine the association between diet and treatment results in patients with RA and SLE.

The outline of this thesis is as follows: After this introduction, Chapter 2 will provide a background of RA and SLE as well as an overview of the previous studies on dietary aspects of the diseases and anti-rheumatic treatments. Chapter 3 will specify the hypotheses and aims of this thesis and Chapter 4 will describe how the methodological procedures were performed in order to answer the specific research questions. Chapter 5 will present the obtained results that are documented in **Papers I-V**. Chapter 6 will discuss and highlight the methodological considerations and main results of **Papers I-V** as well as ethical aspects. After providing some conclusions in Chapter 7, Chapter 8 will list some suggestions for future research based on the main findings. This thesis will end with acknowledgements (Chapter 9) and references (Chapter 10). Copies of **Papers I-V** are listed chronologically at the very end of this thesis.

2 BACKGROUND

2.1 RA

2.1.1 Pathogenesis

RA is a chronic, systemic, inflammatory autoimmune disease of unknown etiology. However, both genetic and environmental factors are contributors to the disease [3]. RA is gender and age related, with the onset more frequent in middle-aged women but it can progress in both women and men at any age. Autoimmunity is the main characteristic of RA, since the immune system is exaggeratedly triggered towards the individual's own healthy cells and even tissues (self-reactive immune response). An overview of the disease development of RA is presented in figure 1.

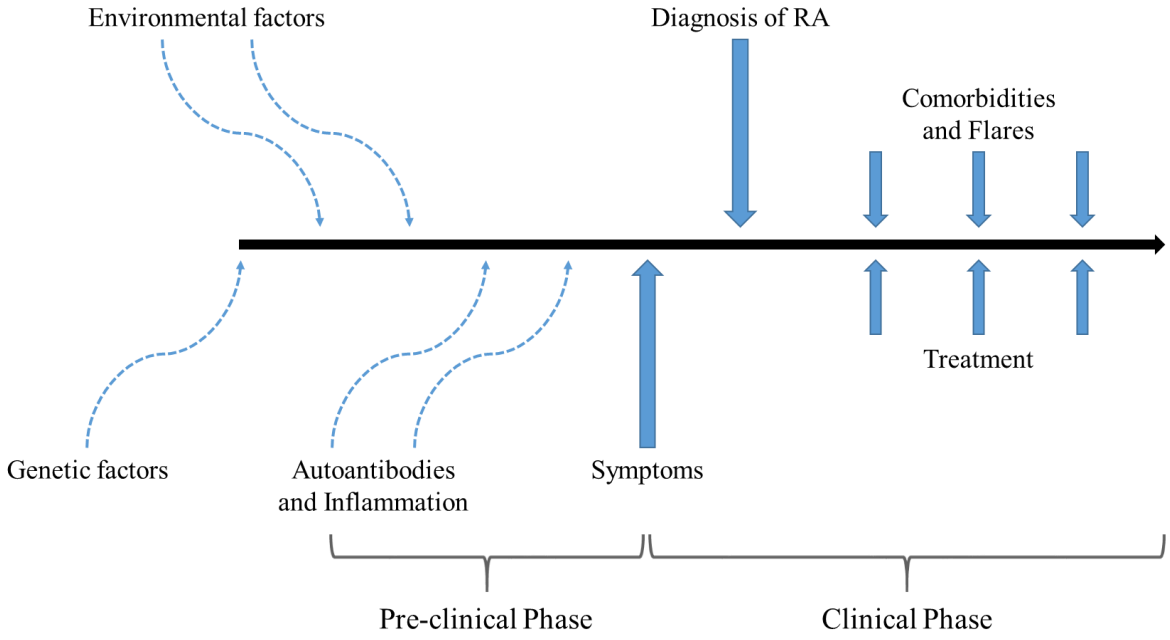

Figure 1. Overview of the disease development of RA. Both genetic and environmental factors may contribute to the pre-clinical phase, during which autoimmunity is triggered followed by inflammatory response. Symptoms start to reveal during the clinical phase and based on these, diagnosis of RA is set. In regards to manifestations as well as the activity and severity of the disease, suitable treatment is initiated for improved disease status.

2.1.1.1 Autoimmunity

In healthy individuals, the immune system has its role to combat infections and prevent tissue damage, where the immune cells are targeting foreign microbes. In contrast, RA patients have an overstimulated immunity leading to uncontrolled production of immune cells that specifically target the body's own healthy cells and tissues.

The autoimmunity of RA stimulates B cell antibody production [4]. The two most studied autoantibodies linked to RA are rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA), which are produced by B cells. The presence or absence of RF/ACPA is referred to as RF/ACPA positive or negative RA, respectively [5]. RF is the classic autoantibody that is directed against the Fc fragment of immunoglobins (Ig); mostly IgM, but also IgA and IgG. RF-Ig complex contributes to the disease process and are regularly found in inflamed joints. RF was first found in RA but is also present in other autoimmune diseases such as SLE, Sjögren syndrome and interstitial pulmonary fibrosis, therefore the RF specificity for RA is low.

In contrast, ACPA has a high specificity for RA and is present in approximately two thirds of RA patients. The two subgroups of ACPA positive and negative RA differ remarkably and can be considered as two separate diseases [5, 6]. Worse prognosis and higher proportions of erosive damage is more common in ACPA positive patients [7, 8]. Smoking is a well-established risk factor for ACPA positivity development [9, 10]. In addition, patients may respond differently to treatment depending on their ACPA status [6, 11].

Infections may activate self-reactive lymphocytes that can trigger the development of autoimmunity. The pathology of RA also stimulates activation of T cells, stromal cells and macrophages that secrete pro-inflammatory cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1 and IL-6, this in turn will attract macrophages, neutrophils and osteoclasts leading to tissue destruction [4, 12].

2.1.1.2 Genetic risk factors

While the source of risk for developing autoimmunity is not fully understood, to and around 50% of factors contributing to RA are genetic [3]. The most significant genetic risk factors for RA are variations in the human leukocyte antigen (HLA), especially the HLA-DRB1 gene in the class II region [13]. HLA-D related (HLA-DR) is a ligand matching T cell receptors. The HLA is a gene complex that encodes cell-surface proteins; major histocompatibility complex (MHC), and is responsible for the regulation of the immune system in humans. MHC, class II

associates well with RA [13]. The presence or absence of HLA has shown to play a major role in the differentiation of ACPA positivity and negativity in RA patients [14].

RA is two to three times more common in women than men. It is found that women are more prone to develop autoimmune diseases overall. Several studies have therefore speculated that the female sex hormones (i.e. estrogen, progesterone) and the X chromosomes affect RA development [15], and the Y chromosome may have a protective effect on autoimmunity development [16].

RA is found in individuals worldwide but the prevalence varies among populations, further that genetics contributes to the etiology of RA [17]. However, a global prevalence of RA is estimated between 0.5 to 1.0% [1], while the prevalence is higher in north European and North American countries compared to South European countries [18]. The highest prevalence of RA has been found in Native American tribes in North America [19], and the prevalence is very low in people from some rural areas in Africa [20].

Twin studies have also shown that genes play a role of the etiology of RA [3]. For instance, a twin affected by RA increases the risk in the other twin by 24.6 to 35.4 times in monozygotic (identical) twins and 17.3 to 31.6 times in dizygotic (non-identical) twins, compared to the general population [21]. Monozygotic twins share 100% of genes and dizygotic twins share 50%.

2.1.1.3 Environmental risk factors

Smoking is the major environmental risk factor for RA [22], particularly in ACPA positive patients [9, 10]. Results from in vitro studies have shown that smoking activates several pro-inflammatory ILs and changes the gene expression in the joints, which can escalate the development of autoimmunity and chronic joint inflammation [14]. In addition, high exposure to silica (mainly through dust) has shown to trigger the development of RA [23, 24].

Body mass index (BMI) may be associated with increased risk of RA. Results from a meta-analysis of observational studies investigating BMI and the risk of RA, results have shown that obesity may increase the risk of RA development. In addition, BMI was also found to be gender related showing that obese women had the highest risk of RA [25].

Diet together with physical activity has a major impact on maintaining a healthy weight or BMI. Thus, an unhealthy diet and sedentary lifestyle may have an influence on the risk of RA. High/regular consumption of sugar-sweetened soda has been associated with increased risk of

seropositive RA in women [26]. There are contradictory results regarding the association between low dietary intake of vitamin D [27-29], high caffeine intake [30, 31] as well as high consumption of red meat [32, 33] and the risk of RA, respectively. Physical activity alone has not shown to reduce the risk of RA [34]. However, physical activity is shown to be beneficial in patient with established RA in regards to quality of life, functionality, pain and number of swollen joints [35].

2.1.2 Manifestations

RA affects primarily joints, but may also affect many other tissues and organs and therefore is considered as a systemic disease. Other organs that could be affected are skin, lungs, heart and blood vessels [36-38]. The clinical manifestations as well as the severity of the disease differ widely between RA patients.

2.1.2.1 Joints

During an autoimmune reaction, the leukocytes (white blood cells) can enter the synovial membrane, which is the lining that protects the joints. This results in destruction of articular cartilage and loss of function of the joints [39]. The affected joints manifest in terms of pain, swelling, tenderness, and deformity. Increased levels of immune cells, such TNF, ILs and anti-producing B cells, are found in inflamed RA joints.

There are several joint count methods for identification of swollen and tender joints that have been developed and validated; the 28-joint count is one of them that is well-implemented in the clinical practice [40]. Swelling and/or tenderness are assessed as present or absent in each joint. Swollen joint count (SJC) and tender joint count (TJC) are objective and subjective measures, respectively. Figure 2 shows the locations of the 28 joints that are examined for the joint count.

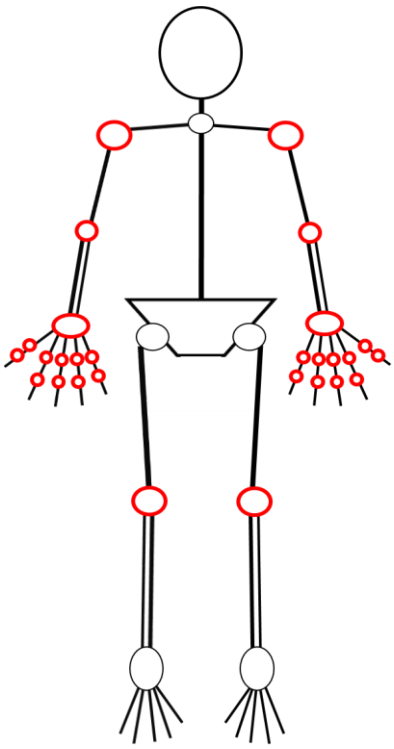

Figure 2. The location of the 28 joints are spotted in red for assessing the 28-joint count, these joints are carefully examined for swelling and/or tenderness.

2.1.2.2 Pain in RA

Pain, mostly in joints, is a dominant and common feature of RA and causes both distress and decreased work capacity [41]. RA patients report pain as a major symptom of their disease and it is found to be a top priority for the patients when starting a treatment. In early disease, pain is usually related to inflammation and correlates well with disease activity [42]. However, many patients report high pain intensity, even during good response to treatment [43, 44]. Thus, earlier data have shown that a subset of patients has significant pain persistence in low disease activity [45, 46]. Pain, fatigue and mood seem to go hand in hand [47]. Personal and environmental factors may also have an impact on pain, for instance gender, age, social background, education, occupation, overall behavior pattern etc. can influence the experience of pain [48]. Pain overall is experienced differently between women and men: when compared to men, women have a lower threshold of pain tolerance and tend to report higher intensity and frequency of pain [49-51]. BMI has been associated with both high disease severity and pain in RA. Consequently, obese RA patients have shown increased pain levels [52-54]. Subjective pain of RA patients may not always correlate with objective clinical measures of the disease [55, 56]. Inflammatory pain in RA is often assumed to reflect a correlation between pain and inflammation. However, pain has also been shown to be weakly correlated to peripheral inflammation [57, 58].

Visual analogue scale (VAS) is a frequently used tool to measure self-reported pain [59, 60] and has been applied in RA patients in order to assess disease related pain. VAS consists of a scale from 0 to 100 millimeter where 0 millimeter indicates *no pain at all* and 100 millimeters *worst pain imaginable* (figure 3).

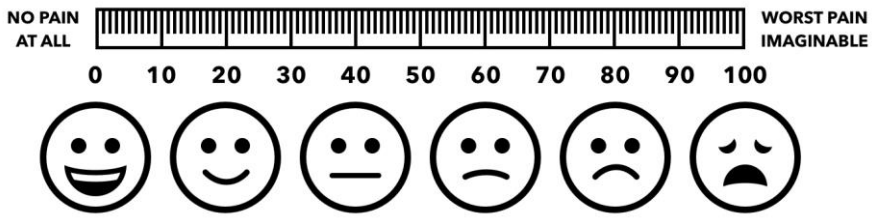

Figure 3. VAS for pain (0-100 mm). Different facial expressions shown below the scale can be used for additional help for children.

2.1.2.3 Additional comorbidities

Comorbidities are common in RA, some examples are cardiovascular diseases (CVD) (including stroke, myocardial infarction, heart failure and atherosclerosis), depression, obesity, diabetes, skin conditions and gastrointestinal ulcers. CVD are associated with increased mortality rate, which explains shorter life expectancy in patients with RA [61]. Osteoporosis is a common comorbidity in RA but is rather considered as a complication of anti-rheumatic treatment, especially of long-term GC treatment [62, 63].

2.1.3 Classification criteria

The American College of Rheumatology (ACR) has developed criteria to classify the diagnosis of RA for which at least four of the seven criteria must be met (table 1) [64].

Table 1. 1987 Classification criteria of RA. At least four of the seven criteria must be met. Criteria 1 to 4 must have been present for at least six weeks. Patients with two clinical diagnoses are not excluded [64].

Criterion	Definition
1. Morning stiffness	Morning stiffness in and around the joints, lasting at least 1 hour before maximal improvement
2. Arthritis of 3 or more joint areas	At least 3 joint areas simultaneously have had soft tissue swelling or fluid (not bony overgrowth alone) observed by a physician. The 14 possible areas are right or left PIP, MCP, wrist, elbow, knee, ankle, and MTP joints
3. Arthritis of hand joints	At least 1 area swollen (as defined above) in a wrist, MCP, or PIP joint
4. Symmetric arthritis	Simultaneous involvement of the same joint areas (as defined in 2) on both sides for the body (bilateral involvement of PIPs, MCPs, or MTPs is acceptable without absolute symmetry)
5. Rheumatoid nodules	Subcutaneous nodules, over bony prominences, or extensor surfaces, or in juxtaarticular regions, observed by a physician
6. Serum RF	Demonstration of abnormal amounts of serum RF by any method for which the result has been positive in <5% of normal control subjects
7. Radiographic changes	Radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify)

MCP, metacarpophalangeal; MTP, metatarsophalangeal; PIP, proximal interphalangeal; RF, rheumatoid factor.

The 1987 Rheumatoid Arthritis Classification has been found to be lacking enough sensitivity for detecting early RA. Therefore, Aletaha D et al. have developed a revised version of these classification criteria; the 2010 ACR/European League Against Rheumatism (EULAR) Rheumatoid Arthritis Classification Criteria (table 2) [65].

Table 2. 2010 ACR/EULAR classification criteria for RA. Target population is 1) patients who have at least one joint with definite clinical synovitis (swelling) and 2) patients with the synovitis not better explained by another disease [65].

Criterion	Score
A. Joint involvement	
1. 1 large joint	0
2. 2-10 large joints	1
3. 1-3 small joints (with or without involvement of large joints)	2
4. 4-10 small joints (with or without involvement of large joints)	3
5. >10 joints (at least 1 small joint)	5
B. Serology (at least 1 test result is needed for classification)	
1. Negative RF *and* negative ACPA 0	0
2. Low-positive RF *or* low-positive ACPA 2	2
3. High-positive RF *or* high-positive ACPA 3	3
C. Acute-phase reactants (at least 1 test result is needed for classification)	
1. Normal CRP *and* normal ESR 0	0
2. Abnormal CRP *or* abnormal ESR 1	1
D. Duration of symptoms	
1. <6 weeks	0
2. ≥6 weeks	1

ACPA, anti–citrullinated protein antibody; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor.

2.1.4 Treatment

Treatment of RA has the aim of providing relief from the symptoms associated with inflammation, pain, stiffness, and of preventing radiological progression that can lead to long-term disability [66]. Important tasks of anti-rheumatic treatment are to inhibit the activation of T cells and the B cell antibody production and/or blocking specific targets such as TNF, IL-1, IL-6 and B cell antibodies. Treatment of RA has advanced remarkably during the last two decades due to improvement of existing drugs but also due to development of new biological drugs.

2.1.4.1 Disease modifying anti-rheumatic drugs

Disease modifying anti-rheumatic drugs (DMARD) can be effective in treating inflammation, delaying joint damage, and improving clinical outcomes of RA [67, 68]. Methotrexate (MTX), which is an anti-folate drug with cytotoxic effect, was first developed for cancer treatment [69], but low dose treatment of MTX became very effective in autoimmune diseases and today it is the most widely used DMARD in RA [70-72]. Folic supplementation in RA patients using MTX is recommended in order to dampen or prevent side effects of the drug [73, 74]. Other commonly used DMARDs for RA are leflunomide, anti-malaria/hydroxychloroquine (HCQ)

and sulfasalazine (SSZ); these drugs, along with MTX are classified as synthetics DMARDs. The most common side effects of DMARDs are manifestations in the gastrointestinal tract and infections.

2.1.4.2 Glucocorticoids

Glucocorticoids (GC) are very common in treating inflammation and are frequently used as in combination with other DMARDs. The anti-inflammatory effect of GC is relatively instant and intense, but long-term GC treatment is associated with several side effects, such as bone loss, osteoporosis [62], hyperglycemia, increased risk of diabetes [75], increased appetite, weight gain, hypertension and risk of atherosclerosis [76-78]. Low dose of GC can be added to the treatment with MTX in order to achieve better outcomes [79]. GC is known to affect metabolism in multiple ways [80, 81]. Low dose prednisolone (drug with predominant GC activity) in RA patients is associated with increased body fat mass [82].

2.1.4.3 Biological drugs

Apart from the synthetic DMARDs mentioned above, new innovative drugs has been developed based on biological compounds that are specifically targeted to TNF, IL, T-cells etc. There are nine approved biological drugs; five of them are TNF inhibitors and four of them are non-TNF inhibitors. All the approved biological drugs are presented in table 3. Combination of MTX and anti-TNF has shown to be an effective anti-rheumatic treatment [83].

Table 3. Biological drugs approved for the treatment of RA [84].

	Drug	**Immunological target**
TNF inhibitor	Adalimumab	TNF
	Certolizumab pegol	TNF
	Etanercept	TNF
	Golimumab	TNF
	Infliximab	TNF
Non-TNF inhibitor	Abatacept	T cell co-stimulation
	Anakinra	IL-1
	Rituximab	B cell
	Tocilizumab	IL-6

2.1.4.4 Assessment of disease activity and treatment results

Disease activity score (DAS) and EULAR response criteria are well-used measures in assessing the disease activity level and assessing response to treatment at specific time point(s),

respectively. DAS28, which is a modified DAS based on the 28 joints, is the most widely used measure for disease activity in RA. It is obtained through a formula which includes number of swollen and tender joints, erythrocyte sedimentation rate (ESR) (or C-reactive protein (CRP)), and patient's global assessment of overall health (through VAS). Based on DAS28, different disease activity levels of RA can be categorized into remission as well as low, moderate and high disease activity (figure 4). Higher DAS28 is positively correlated with degree of inflammation. [85-87]

Figure 4. Disease activity levels of DAS28 in RA.

Based on DAS/DAS28, EULAR response was developed in order to evaluate treatment outcome by examining the change of DAS28 from treatment initiation/baseline as well as DAS28 at endpoint/follow-up. It is a validated tool for assessing treatment efficacy in RA [88-90]. EULAR response is categorized into none, moderate and good response (table 4).

Table 4. EULAR response criteria based on DAS28. [88]

DAS28 at endpoint:	**Improvement in DAS28 from baseline:**		
	>1.2	>0.6 and ≤1.2	≤0.6
≤3.2	Good response	Moderate response	Non-response
>3.2 and ≤5.1	Moderate response	Moderate response	Non-response
<5.1	Moderate response	Non-response	Non-response

2.1.5 Diet in RA

Certain specific diets, such as vegetarian, Mediterranean, vegan and gluten free diet, have been shown to some extent to ameliorate the disease course of RA [91-96]. The Mediterranean diet encourages increased intake of vegetables, fruits, legumes, fatty fish and olive oil, and reduced/moderate intake of red meat and dairy products [97]. High consumption of olive oil and fatty fish (oil) has shown to give a protective effect of RA [98, 99]. Increased fruit and vegetable intake may reduce the risk of RA [100-102], and may also be beneficial for prevalent RA patients [103, 104]. In contrast, a dietary intake high in caffeine, low in antioxidants and high in red meat may increase the risk of RA [105].

Dietary micronutrients could play an important role in RA. Free radicals, produced by activated macrophages, monocytes and granulocytes, have been found in damaged inflamed tissues and synovial fluid [106-109]. Intake of antioxidant micronutrients, such as vitamins A, C, and zinc, have been found to be insufficient in patients with RA and may affect joint damage and loss of rheumatic function. Increased antioxidant intake may reduce inflammation in RA [110]. TNF has also shown to be inhibited by antioxidants [108, 111]. It has been speculated that anti-inflammatory treatments of RA may have an additional role of antioxidant effect. Therefore, micronutrients with antioxidant properties might be beneficial during tissue damaging in RA, and can act as a complementary therapy [106].

Vitamin D is involved in various aspects of inflammation and immunity and is one of the micronutrient that has been recently most studied in RA due to its anti-inflammatory effect. Vitamin D levels are found to be decreased in patients with RA, this has also been reported in RA patients in Sweden [27, 112-115]. Some evidence suggest that vitamin D deficiency can trigger autoimmune responses and therefore, vitamin D may provide an immunoregulatory effect [116, 117]. Vitamin D deficiency has also been associated with increased risk of RA [27]. The active metabolite of vitamin D ($1,25(OH)_2D$) inhibits the synthesis of ILs such as IL-1, IL-6, IL-12 and TNF through macrophages. It also decreases MHC-II expression on cell surface molecules such as cluster of differentiation (CD)86, CD80 and CD40 [118]. Vitamin D supplementation has shown to decrease the disease activity in short-term in RA patients [119], and several studies have suggested that vitamin D supplementation may be beneficial in RA, in order to reduce inflammation or disease activity [120-124].

Omega-3 and omega-6 fatty acids (FA) are polyunsaturated FA (PUFA) that have been associated with inflammation. Earlier data suggest that omega-3 FA are anti-inflammatory and can decrease disease activity in RA [125-128]. Also, excessive intake of omega-6 FA as well

as increased omega-6 to -3 FA ratio have been linked with pro-inflammatory properties in RA [129]. Increased dietary intake of omega-3 FA has been associated with decreased serum levels of tumor TNF and C-reactive protein (CRP) in RA [130]. On the other hand, higher intake of omega-6 FA has been associated with increased serum levels of IL-6 and CRP in RA [130]. Important derivatives of omega-3 FA are resolvins, protectins and lipoxins. These non-classical eicosanoids have anti-inflammatory properties, and resolvins have shown to be linked with reduction of inflammatory pain [131, 132]. Resolvins dampen indirectly the production of pro-inflammatory omega-6 FA derived eicosanoids, such as leukotrienes and prostaglandins, by inhibiting the enzymes lysyl oxidase and cyclooxygenase 1 and 2 [133]. Therefore, it is important to have a good balance between omega-6 and omega-3 [134]. High consumption of fatty fish containing omega-3 FA may reduce the inflammation compared to high consumption of red meat.

Niacin is involved in energy metabolism and is also important in cell division. Higher lipoprotein levels are found in patients with RA [135, 136]. A study done by Villines TC et al. has shown that niacin reduces low density lipoprotein (LDL) cholesterol, very low density lipoprotein cholesterol, and triglycerides, but effectively increases high density lipoprotein cholesterol [137].

Non-iron deficient anemia (anemia of chronic disease) is commonly seen in patients with RA. Inflamed tissues secrete IL-1 and IL-6, which affect iron metabolism, bone marrow, and erythropoietin production by the kidneys. Iron supplementation is effective in correction of anemia, as well as in reduction of disease activity in RA patients [138]. However, more studies with similar results are needed to support the understanding of the beneficial effect of higher iron intake in RA.

Several studies have indicated that alcohol intake in moderation may have a protective effect of RA development [139-141]. From a meta-analysis regarding alcohol intake and the risk of RA, results showed that low to moderate alcohol consumption was inversely associated with RA development in a dose-response manner, it was also reported that an alcohol intake exceeding 15 g per day did not raise any further beneficial effect. [142]. The beneficial effect of alcohol may be due to downregulation of immune response [143] as well as decreased production of pro-inflammatory cytokines [144, 145].

2.2 SYSTEMIC LUPUS ERYTHEMATOSUS

2.2.1 Pathogenesis

Systemic lupus erythematosus (SLE), like RA, is a chronic, systemic, inflammatory autoimmune disease with unknown cause that occurs primarily in women (approximately 90% [146]) and can involve any organ. Symptoms of SLE symptoms usually debut between ages of 15 and 45 years, but can occur earlier or later in life as well. Compared to RA, the disease activity of SLE can vary more between flares and/or remissions [147]. The global prevalence of SLE differs between age, gender and ethnic groups [148], and is estimated to be 3-200 per 100,000 people [2].

2.2.1.1 Autoimmunity

The autoimmunity of SLE involves abnormal activation of immune cells, mainly of dysregulated B cells, inflammatory T cells. In SLE, the autoimmune response can be scattered throughout the body, which can cause inflammation that affect multiple organs and systems. SLE can be characterized by production of common autoantibodies, there are more than 100 autoantibodies that have been seen in SLE patients, however, all of these have not been considered as pathogenic and it is unclear whether all of them contribute to the disease [149]. This characterization is somewhat different to other rheumatic diseases in regards to number of shared autoantibodies. The most common autoantibody associated with SLE are anti-nuclear antibody (ANA), anti-double stranded deoxyribonucleic acid (dsDNA) and anti-Smith (Sm). ANA is found in almost all SLE patients (90-100%) and anti-dsDNA and anti-Sm are found in the vast majority of SLE patients [149]. SLE patients are known to have increased levels of pro-inflammatory cytokines; interferons, IL-6, IL-10 and TNF. Interferons have been associated with disease activity and severity of SLE. The disease course and manifestations can differ depending on the presence of different antibodies.

2.2.1.2 Genetic risk factors

Like RA and many other autoimmune diseases, the HLA gene is strongly associated with SLE. Many studies have explored this gene in relation to the risk of SLE in different ethnic groups and have concluded that the HLA region is the strongest predictor of genetic risk [150]. The genes of HLA-DRB1 (especially DRB1*1501 and DRB1*0301) expressed by HLA, class II have been robustly associated with SLE [151]. Genetic complement deficiency of complement proteins is rare but strongly associated with the disease development.

The global prevalence of SLE is estimated to be 3-200 per 100,000 people [2], but differs between different ethnic groups as well as age and gender [148]. The SLE incidence is strongly related to ethnicity, for instance, the rate is four times higher in African-Americans than in European-Americans [152]. Interestingly, SLE is rare in Africa [153], but people with African (and Asian) descent residing outside Africa have two to three higher incidence and prevalence rates as well as increased disease severity than Caucasians [154]. Higher rates have also been seen in Aborigines [155] and in some Native American groups [156].

First-degree relatives (offspring, sibling or parent) of SLE patients have 20-fold greater risk for the disease than the general population [152, 157]. In addition, a monozygotic twin sister or brother has approximately ten times higher risk to develop SLE than in the same comparison to dizygotic twins [158, 159].

SLE can be called the "women's disease" because of the female dominant prevalence. Incomplete inactivation of the second X chromosome can lead to overexpression of X-linked genes (i.e. FOX, TNF and TLR7) and micro ribonucleic acid that have been associated with increased risk of SLE [160, 161]. In addition to genetic factors, female sex hormones (i.e. estrogen and progesterone) can also have an impact on the disease development [15].

2.2.1.3 Environmental risk factors

SLE can be triggered by smoking, ultraviolet light exposure, infections (i.e. Epstein Barr virus, parvovirus (EBV)), silica dust, pollution and contraceptive use.

Current smokers have been shown to have 50% increased odds of developing SLE, compared to non-smokers in a meta-analysis [162]. Smoking may trigger autoimmunity by generating free radicals inducing inflammation [163].

UV radiation have been linked to increased DNA damage of free radicals, production of autoantigens and autoreactive T cells, immunomodulatory effects on T cells and cytokines, which all involve in the pathogenesis of SLE [164].

Several studies have shown that high EBV load is remarkably increased and has been associated with the development of the disease [165]. Results from a study performed in Taiwan showed that serum levels of EBV DNA are remarkably elevated in SLE patients (42%), compared to healthy controls (3%) [166].

Animal studies have shown that exposure to silica dust and air pollution may trigger autoimmunity [167]. However, similar findings in humans are lacking.

The Nurses' Health Study has reported that oral contraception is associated with onset of SLE [168]. However, other studies with focus on oral contraception and risk of SLE have shown contradictive results [169].

Additional potential risk factors are socioeconomic status, cosmetics use, vaccination and stress [170].

2.2.2 Manifestations

SLE can be called "the great imitator" due to the heterogeneity of the disease by sharing many manifestations of other diseases. SLE patients often present with general systemic inflammation with specific manifestations involving the heart, joints, skin, lungs, blood vessels, liver, kidneys, and nervous system. [171, 172]. The diversity of clinical manifestations in SLE can range from mild arthritis through to pericarditis and nephritis to life-threatening neuropsychiatric manifestations [173]. Some of the common manifestations/complications will be discussed below.

2.2.2.1 General symptoms

General symptoms include fever, fatigue, reduced appetite and weight loss. Chronic fatigue is expressed in many patients and even severe fatigue during flares. In addition to disease related inflammation, anemia, heart and lung manifestations, psychological problems, sleep deprivation and treatment side effect can also increase fatigue. Exercise have shown beneficial effect to reduce fatigue in SLE patients, compared to controls [174].

2.2.2.2 Musculoskeletal system

Inflammation and pain in joints are seen in approximately 50% of people who start to develop SLE and in more than 90% in patients with established SLE. However, affected joints in SLE are less severe than in RA. Inflammation in muscles (myositis), stiffness and unspecific pain are seen in 25% of SLE patients.

2.2.2.3 Skin

Lupus in Latin means "wolf" due to the characteristic facial rash, also called butterfly rush, in SLE patients. *Erythematosus* is related to the redness of the rash seen in SLE. The skin

manifestation in SLE can be divided into 1) chronic cutaneous (discoid), 2) subacute cutaneous and 3) cute cutaneous. Skin manifestation are seen in approximately two thirds in SLE patients , these can cause rashes or sores (lesions) and most of them are developed on body parts such as the face, ears, neck, arms and legs during sun exposure. Smoking increases the risk of SLE related skin damage [175]. Examples of skin related conditions in SLE are cutaneous vasculitis lesions (inflamed blood vessels in the skin, often appears as red-purple spots, bumps and even ulcers), Raynaud's phenomenon (restricted blood flow in hands) and hair loss. Avoiding smoking, sun exposure and using sunscreen are of importance in SLE [176].

2.2.2.4 Kidneys

When the glomeruli in kidneys become inflamed, lupus nephritis (LN) can occur. LN affects approximately 50–70% of SLE patients and is a major cause for increased morbidity and mortality [177, 178]. There are five types of LN (class I-V) that have been developed based on different part or function that have been affected as well as the severity of LN. Histological classification of LN has been developed by the International Society of Nephrology/Renal Pathology Society (ISN/RPS) and is presented in table 5 [179].

Table 5. ISN/RPS 2003 classification of LN [179].

Class	Description
I	Minimal mesangial LN
II	Mesangial proliferative LN
III	Focal LN
IV	Diffuse LN
V	Membranous LN
VI	Advanced sclerosis LN

2.2.2.5 CVDs

SLE patients have increased prevalence of carotid atherosclerotic plaques and the risk of developing CVD, compared to healthy controls [180-185]. Atherosclerosis is a main leading cause of CVD [185]. Some well-known risk factors of CVD are age, male gender, smoking, family history, diabetes mellitus and hypertension. It has been speculated that diet plays a role in atherosclerosis in SLE and it has been shown that certain dietary micronutrients (riboflavin, phosphorus, selenium and thiamin) may be inversely associated with atherosclerotic plaque in SLE patients [118] (**Paper II**).

2.2.3 Classification criteria

There is no "gold standard" test for the diagnosis of SLE. However, the diagnosis is usually based on characteristic symptoms, signs and laboratory findings. ACR has developed criteria to classify the diagnosis of SLE for which at least four of the eleven ACR criteria must be met (table 6) [186].

Table 6. The 1982 Revised Criteria for Classification of Systemic Lupus Erythematosus. Any four or more of the eleven criteria must be present, serially or simultaneously during any interval of observation [186].

Criterion	Definition
1. Malar rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
2. Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions
3. Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
4. Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by physician
5. Arthritis	Nonerosive arthritis involving 2 or more peripheral joints, characterized by tenderness, swelling, or effusion
6. Serositis	a) Pleuritis--convincing history of pleuritic pain or rubbing heard by a physician or evidence of pleural effusion *OR* b) Pericarditis--documented by ECG or rub or evidence of pericardial effusion
7. Renal disorder	a) Persistent proteinuria greater than 0.5 g/day or greater than 3+ if quantitation not performed *OR* b) Cellular casts--may be red cell, hemoglobin, granular, tubular, or mixed
8. Neurologic disorder	a) Seizures--in the absence of offending drugs or known metabolic derangements; e.g., uremia, ketoacidosis, or electrolyte imbalance *OR* b) Psychosis--in the absence of offending drugs or known metabolic derangements, e.g., uremia, ketoacidosis, or electrolyte imbalance
9. Hematologic disorder	a) Hemolytic anemia--with reticulocytosis *OR* b) Leukopenia--less than 4,000/mm^3 total on 2 or more occasions *OR* c) Lyphopenia--less than 1,500/mm^3 on 2 or more occasions *OR* d) Thrombocytopenia--less than 100,000/mm^3 in the absence of offending drugs
10. Immunologic disorder	a) Positive LE cell preparation *OR* b) Anti-DNA: antibody to native DNA in abnormal titer *OR* c) Anti-Sm: presence of antibody to Sm nuclear antigen *OR* d) False positive serologic test for syphilis known to be positive for at least 6 months and confirmed by *Treponema pallidum* immobilization or fluorescent treponemal antibody absorption test
11. Antinuclear antibody	An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs known to be associated with "drug-induced lupus" syndrome

2.2.4 Treatment

Treatment strategy is based on the location and severity of the disease related flare. SLE has previously been considered as a life-threating disease due to its severe complications, but due to improved treatment development, the mortality has decreased remarkably. The main focus of treating SLE is to suppress the inflammation. Common treatments used for SLE are GC, anti-malaria/HCQ, non-steroid anti-inflammatory drugs (NSAIDs) and immunosuppressive agents [187]. Recently, biological drugs have also become used for treating specific SLE manifestations.

2.2.4.1 GC

GC is the most effective anti-inflammatory treatment for chronic inflammation [188], the anti-inflammatory effect of GC is obtained when GC binds to the glucocorticoid receptor, which has pro-inflammatory properties alone. When the GC complex gets activated, it upregulates the expression of anti-inflammatory proteins in the cytosol [189-191]. Although GC is very effective in treating inflammation, it cannot be ignored that long-term GC treatment comes with several side effects [77, 78]. The disease activity levels in SLE patients may be distinguished in regards to GC treatment (**Paper II**) [192]. GC has been associated with higher disease activity in SLE. [193, 194]

2.2.4.2 Anti-malaria

Anti-malaria/HCQ is widely used in SLE patients, especially those with arthritis and/or skin manifestations. Additional reported benefits of HCQ in SLE patients are lowered levels of total cholesterol, improved insulin sensitivity in obese non-diabetic patients and reduced occurrence of thrombotic events [195]. HCQ is also safe during pregnancy [196].

2.2.4.3 Immunosuppressive drugs

Immunosuppressive drugs that are commonly used in SLE are cyclophosphamide (CYC), azathioprine (AZA), mycophenolate mofetyl (MMF) and MTX. Many of them are combined with GC in order to obtain better treatment outcome. CYC, together with GC, is usually used for neuropsychiatric manifestations, severe and/or life threatening SLE manifestations as well as for induction therapy of LN [195]. AZA and MMF combined with GC, respectively, are often used as maintenance therapy [197]. AZA has shown to have a GC sparing effect in SLE patients [195], the same effect has been seen for MMF (**Paper VI**) [27]. MTX is more used for treating RA but has also been used for SLE patients with mainly arthritic and cutaneous

manifestations [197]. Common side effects of oral immunosuppressive drugs are manifestations in the gastrointestinal tract.

2.2.4.4 Biological drugs

There are several biological drugs used in SLE with different blocking targets such as B cells, T cells, cytokines and innate immunity. Examples of biological drugs are presented in table 7 [198]. It is important to underline that only belimumab is approved by Food and Drug Administration for treating SLE, the other ones are used as "off-label". Belimumab has shown to be effective for treating LN [199], and sifalimumab has shown promising results in terms of global and organ-specific measures of disease activity [200]. Biological treatment is decided depending on observed specific manifestation in patients and/or the main blocking immunological target of interest.

Table 7. Biological drugs used in SLE [198]. Belimumab is the only one approved for SLE, the others are used as "off-label".

	Drug	**Immunological target**
B cell	Abatacept	CD80/86
	Belimumab	BLyS
	Epratuzumab	CD22
	Ocrelizumab	CD20
	Rituximab	CD20
	Sifalimumab	INF
T cell	Tocilizumab	IL-6
	Sirukumab	INF

BLyS, B lymphocyte stimulator; CD, cluster of differentiation; INF, interferon; IL, interleukin.

2.2.4.5 Disease activity assessment

The disease course of SLE varies with flares and is often unpredictable. There are several tools for measuring the disease activity, two measures that are frequently used are SLE disease activity index (SLEDAI) [201] and systemic lupus activity measure (SLAM) [202, 203] and British isles lupus activity group (BILAG) [204]. These measures are based on expressed manifestations, laboratory measures and/or physician's and patient's global assessments. Another measure that focuses on organ damage in SLE is systemic lupus international collaboration clinics (SLICC), this measure can be useful when looking at long-term outcomes [205].

2.2.5 Diet in SLE

Little is known about the dietary aspects of in SLE. Disease condition as well as the treatment(s) may weaken the nutritional status in patients with SLE, compared to the general population. In addition, dietary habit or nutritional status may also influence the severity of the disease as well. For instance, zinc and selenium levels in serum have been found in SLE patients, compared to healthy controls [206]. A study on nutritional status and food intake in 170 women with SLE showed an inadequate calcium intake and low consumption of fruits and vegetables in the majority of the patients [207]. Higher intake of vitamin B_6 and dietary fiber may prevent the occurrence of active disease in Japanese SLE patients [208]. Fish oil (omega-3 FA) has shown in several studies to have an anti-inflammatory effect and it has been reported some beneficial effect for SLE patients [209]. Mediterranean diet can delay the progression of internal carotid intima media thickness (IMT) and plaque height in women (aged 60-80 years) and men (aged 55-80 years) with high risk of cardiovascular events [210]. Elkan AC et al. have studied the macronutrient intake in SLE patients and healthy controls and reported that SLE patients had decreased intake of PUFA and fiber as well as increased intake of carbohydrates, compared to the controls [211]. In an animal study of mice induced with autoimmune nephritis, a diet increased in omega-3 FA intake was associated with reduced expression of several pro-inflammatory components (i.e. CD80, IL-10, IL-18, IL-6, TNF) in kidney and/or spleens, compared to diets rich in omega-6 FA or omega-9 monounsaturated FA [212]. Excessive protein intake can alter bone mineral loss in juvenile SLE [213]. A diet rich in protein may not be recommended for patients with SLE, especially in LN patients since nitrogen imbalance in kidneys can occur. Protein-restricted diet (less than 0.6 kg per day) has shown to improve the glomerular filtration rate in chronic kidney disease of patients with systemic diseases [214, 215]. Previous findings suggest that vitamin D and moderate alcohol intake are beneficial for SLE patients. Vitamin D deficiency has been linked with higher disease activity [216-218], and moderate alcohol consumption has shown to be associated with reduced risk of SLE [219-221].

2.3 DIET AND TREATMENT

2.3.1 Nutrient-drug interaction

Dietary nutrients may have an impact on treatment effectiveness depending on how the oral treatment is consumed. Generally, oral treatment is to be taken during food intake in order to avoid upset stomach, and an empty stomach can also interfere with the way the medicine is absorbed. In contrast, some oral treatment may inhibit the nutrient absorption and therefore it would be more effective if drugs and food were taken separately. Several nutrients and drugs interact and these nutrient-drug interactions can lead to nutrient imbalances or interfere with drug effectiveness [222]. This may take place in different ways; 1) drugs may change the absorption, metabolism and excretion of nutrients and 2) foods and nutrients may change the absorption, metabolism and excretion of drugs. Patients with chronic diseases, such as RA and SLE, might be experiencing important adverse nutrient-drug interactions since drugs are taken over longer periods. There is a lack of studies that have specifically investigated the influence of diet on treatment results in patients with RA and SLE.

2.3.2 Diet and MTX in RA

MTX is the most widely used DMARD in RA [70-72] and a well-known nutrient-drug interaction occurs between MTX and folate since MTX is a folate antagonist [223]. Folate stores are decreased in RA patients treated with MTX. Impaired folate status is also related to MTX toxicity. Gastrointestinal intolerance is one of the side effects of MTX, and folic acid and folinic acid supplementation have been shown to reduce the mucosal and gastrointestinal side effects during low dose of MTX treatment [73, 224]. Although MTX is believed to work through folate antagonism, it has also been hypothesized that the anti-inflammatory effect of this drug might be due to its stimulation of adenosine release [225-227]. Folic supplementation in RA patients using MTX is recommended in order to dampen or prevent side effects of the drug [73, 74].

Evidence suggests that long-chain omega-3 FAs are beneficial in the treatment of autoimmune and inflammatory conditions [228, 229]. Combination of MTX and omega-3 have shown a significant reduction in liver enzyme activities [230]. Anti-inflammatory effect of omega-3 FA may be beneficial before treatment initiation of MTX, and therefore combination of omega-3 FA and MTX might enhance the treatment efficacy of MTX in RA (**Paper I**). In addition, cod liver oil supplements containing omega-3 FA can be used as sparing agent of NSAIDs in RA patients [231, 232].

Vitamin B_6 and vitamin B_{12} work as co-factors the involvement of folic acid in DNA synthesis and methylation, as well as a regulator of homocysteine [233]. Patients with RA are found to have an increased level of homocysteine [234-236], and that these levels may be due to side effects of MTX treatment [237, 238], although, other studies do not show this relationship [235, 236]. Studies have reported low levels of circulating vitamin B_{12} and B6 in RA patients [239, 240], and that homocysteine levels in RA patients can successfully be decreased by vitamin B supplementation [241].

Vitamin E is a biological antioxidant that has found to have a role in controlling chronic inflammation of any cause [242]. Low levels of vitamin E, β-carotene (vitamin A), and selenium in combination may be a risk of RA [243]. Similar results have been shown with lower serum concentrations of α-tocopherol, β-carotene, and retinol [244]. Vitamin E supplementation accompanied with MTX and SSZ in RA patients showed an increased levels of glutathione peroxidase (antioxidative) than in the control group without vitamin E supplement [245]. There might not be a direct link between vitamin E and MTX, although recommended daily intake (RDI) of vitamin E may be beneficial for overall health in patients with RA during MTX treatment.

Retinol (vitamin A) alone has been found to protect small intestines from side effects of MTX in animal studies [246-248], but similar findings based on human data are lacking. Thus, the link between vitamin A and MTX in RA remains unclear.

Selenium is an essential trace element in human and animal nutrition and has an antioxidant effect on regulation of thyroid hormone metabolism [249]. Selenium has a role in the formation and function of the selenium-dependent glutathione peroxidases, enzymes that protect from oxidative damage [250]. Restoration of glutathione peroxidase activity by selenium supplement can prevent lipid peroxidation in animals with selenium deficient diet, thus providing the protective effects against liver damages [251]. Low selenium concentrations in serum have been reported in RA patients and particularly in the Nordic countries [252-254]. Other studies have shown that lower levels of selenium in serum is associated with an elevated risk of RA [243, 244]. Combination treatment of MTX and selenium significantly improves liver function through hepatoprotection against MTX induced hepatotoxicity in rabbits [230], but this has not been studied in humans.

Turmeric is a deep orange-yellow powder spice of the ginger family. Curcumin is an important fraction of turmeric and has been suggested to have an anti-rheumatic effect with anti-inflammatory, anti-hyperlipidemic, anti-oxidant, anti-viral, and anti-microbial activity [255,

256]. Concomitant intake of MTX and curcumin in rats has shown a significant anti-arthritic action and protection from hematological toxicity [257].

2.3.3 Diet and treatment in SLE

The link between diet and treatment results in SLE is rather unknown. GC treatment is frequently used to control active SLE. Older studies have shown reduced intestinal calcium absorption and reduced vitamin D levels during GC treatment [258, 259]. In addition, GC has shown to be associated with increased appetite and/or body weight [260]. Increased appetite during GC treatment have been self-reported as one of the major adverse events in several studies [261-263]. Vitamin D and calcium supplementation is highly recommended during GC treatment [264].

As mentioned earlier, patients with SLE have higher risk for vitamin D deficiency due to lower sun exposure and use of sunscreen. In addition, HCQ has shown to have an inhibitory activity in conversion of vitamin D to its active form ($1,25(OH)_2D$) in SLE patients [265], and therefore, vitamin D levels may be decreased due to HCQ treatment.

MMF has shown to improve the lipid profile in SLE induced mice, after twelve weeks of high cholesterol Western diet; mice who received MMF had decreased atherosclerotic lesions compared to the control group without MMF [266]. However, the association between MMF and iron is unclear. One study claims that iron decreases the MMF absorption in healthy individuals during concomitant intake of MMF and iron [267]. Another similar study showed no relation between MMF and iron [268].

3 HYPOTHESES AND AIMS

3.1 HYPOTHESES

This thesis is based on five hypotheses regarding different aspects of diet and treatment results in RA and SLE:

1. Specific dietary nutrients may associate with response to anti-rheumatic treatment in patients with RA.
2. Diet may associate with disease activity/GC treatment in patients with SLE.
3. Women who have been diagnosed with RA may change their diet in the long run.
4. PUFA intake may associate with pain patterns in patients with RA.
5. Dietary micronutrient intake may associate with atherosclerosis in patients with SLE.

3.2 SPECIFIC AIMS

The overall aim of this thesis was to investigate the association between dietary factors and treatment results in patients with RA and SLE, and the specific aims were to study:

1. the association between dietary intake of vitamin D, omega-3 FA, folate and EULAR response in DMARD treated early RA patients (**Paper I**).
2. the association between diet and GC treatment in patients with SLE (**Paper II**).
3. the long-term dietary changes in women after RA diagnosis (**Paper III**).
4. the association between dietary PUFA and pain patterns in MTX treated early RA patients (**Paper IV**).
5. the association between micronutrient intake and atherosclerotic plaque in patients with SLE (**Paper V**).

4 MATERIALS AND METHODS

4.1 REGISTERS

This thesis is based on data of participants from three registers; Epidemiological Investigation of Rheumatoid Arthritis (EIRA) (**Paper I**, **Paper IV**), SLE Vascular Impact Cohort (SLEVIC) (**Paper II**, **Paper V**) and Swedish Mammography Cohort (SMC) (**Paper III**). EIRA and SMC have been linked to the Swedish Rheumatology Quality register (SRQ) in order to provide clinical data of patients with RA. All participants from the three registers were asked to complete food frequency questionnaires (FFQ) regarding their dietary habits.

4.1.1 EIRA

4.1.1.1 Outline

EIRA is an ongoing population based case-control study that aims to investigate possible genetic and environmental risk factors of RA. EIRA was initiated in 1996 and aimed to recruit newly diagnosed RA patients fulfilling the 1987 ACR criteria for RA, aged 18-70 years, as well as randomly selected healthy controls (two controls per patient) from the general population that were matched by age, gender and area of residence. All the participants who entered EIRA were asked to complete a questionnaire regarding life style factors, occupational exposures, health aspects, socio-economic factors and demographic data. EIRA initially distributed the questionnaire at inclusion/baseline but eventually started to distribute follow-up questionnaires after one year and three years. The original baseline questionnaire (EIRA, part I) has been updated with further detailed questions (EIRA, part II). The response rate for EIRA, part II was 92% and 73% among RA patients and controls, respectively. In addition, participants have given blood samples for further laboratory investigations of disease status and genetic factors. The EIRA study is based at Institute of Environmental Medicin (IMM), Karolinska Insitutet Solna, Stockholm, Sweden.

4.1.1.2 Dietary assessment

EIRA, part II includes a FFQ of participants' dietary habits. This self-administered, semi-quantitative FFQ included questions regarding frequency intake of 123 food items and beverages during the previous year before baseline. Pre-specified food frequency intake ranged over eight categories from *Never* to *≥3 times per day*. For frequently consumed foods, open questions were used and the participants could fill in number of slices, cups, glasses etc. The respondents could also specify their usual portion sizes (i.e. small, medium, large in relation to

pre-specified medium size). Based on the reported food frequency intake of the study participants, an estimated average daily intake of several nutrients were calculated. The FFQ also included questions regarding supplement use of vitamin D, omega-3 FA/fish oil and folic acid, however, supplement use was not included in the calculation of nutrient intake.

In **Paper I**, dietary nutrient intake of vitamin D (µg per day), omega-3 FA (g per day) and folate (µg per day) were calculated by multiplying the average frequency of consumption of each food item by the nutrient content of age and gender specific portion sizes [269]. Total omega-3 FA intake was calculated based on the most common FA of omega-3 ((alpha-linolenic acid, C18:3/10) + eicosapentaenoic acid, C20:5 + docosapentaenoic acid, C22:5 + docosahexaenoic acid, C22:6) [270, 271]. All dietary nutrients were energy adjusted to the mean of the total energy intake (1 938.90 kcal per day) in the study population, using the residual method [272]. The dietary assessment method and the validation of this FFQ have been described previously [273, 274].

For **Paper IV**, dietary data of omega-3 FA and omega-6 FA intake were used. Omega-6 FA intake was calculated by summing linoleic acid (FA C18:2) and arachidonic acid (FA C20:4). Based on the PUFA intake, omega-6 to 3 FA ratio were calculated.

The estimated vitamin D intake was validated in regards to 4 x 1-week weighed dietary records of vitamin D intake (n=129). Pearson's correlations of FFQ-vitamin D intake was 0.60–0.70 for different types of vitamin D–fortified reduced-fat dairy products, 0.30–0.70 for vitamin D–fortified margarines, and 0.50 for different types of fatty fish [275]. Estimate of FFQ based omega-3 FA intake has been validated in comparison to adipose tissue composition (n=239) and 4 x 1-week weighed dietary records of total polyunsaturated fatty acid (PUFA) intake (n=184), respectively. Pearson's correlations were 0.41 between the estimated intake of omega-3 FA and adipose tissue [276] and 0.40 between the estimated intake of omega-3 FA and dietary records of total PUFA intake [277]. Pearson's correlations was 0.50 between the estimated intake of folate and 4 x 1-week weighed dietary records of folate intake (n=129) [278].

4.1.1.3 Assessment of demographic and lifestyle factors

Paper I and **Paper IV** used additional data such age, gender, BMI (weight (kg)/[height (m)]^2), smoking history, education and physical activity. Smoking status was categorized into never, former and current smoker for assessment of baseline characteristics and smoking pack year(s) was used for analysis adjustments and was categorized into 0–9, 10–19 and 20 pack years; one pack year was equal to 20 smoked cigarettes per day during one year [279]. Education level

was categorized into high school and university degree. Performed physical activity during the previous year before baseline was categorized into four levels; sedentary, moderate occasional and moderate regular physical activity as well as regular exercise.

4.1.1.4 Outcome assessment

In **Paper I**, the outcome was treatment result of DMARDs, which was measured through EULAR response after three months. EULAR response was based on DAS28 at three months as well as changes of DAS28 from baseline to three months. The calculation of the EULAR response criteria is presented in table 4. EULAR response was dichotomized into non- to moderate response versus good response.

In **Paper IV**, the outcome was non-inflammatory pain after three months of MTX treatment, which reflected pain in spite of inflammatory control. This type of pain was defined as Refractory Pain based on VAS for pain (0-100 mm) together with CRP level (mg/L). Patients Acceptance Symptom State (PASS) is a validated measure that indicates the level of acceptable pain, among other clinical measures [280]. According to PASS, a VAS for pain above 40 mm indicates *unbearable pain* or *unacceptable pain*. Inflammatory control was set at CRP below 10 mg/L [281]. Refractory Pain was specifically defined as "VAS pain above 40 mm and CRP below 10 mg/L". The outcome measure was with versus without Refractory Pain.

4.1.1.5 Study participants

EIRA, part II provided data on 1 296 RA patients, fulfilling the 1987 ACR criteria and 2 632 matched controls. The inclusion period for **Paper I** and **Paper IV** was during October 2005 to March 2012. An overview of the participant selection is presented in figure 5.

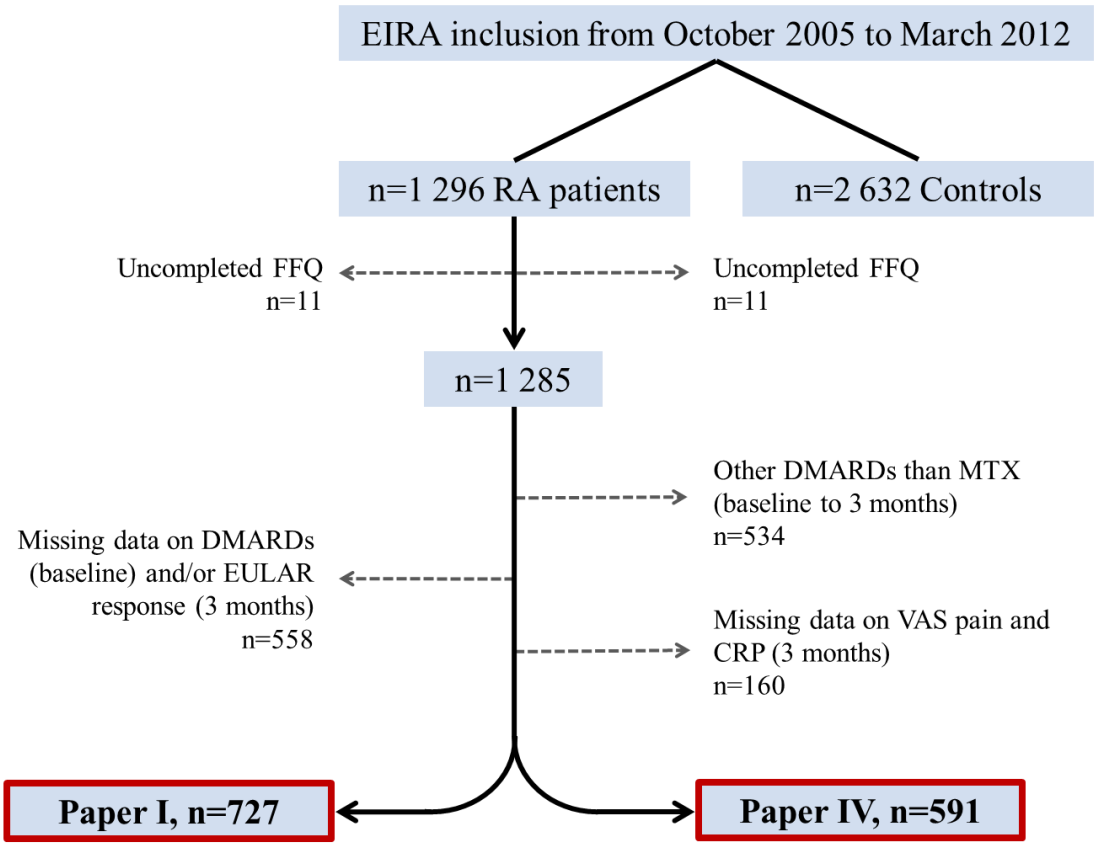

Figure 5. Overview of participant selection in **Paper I** and **Paper IV**, respectively.

4.1.1.6 Statistical analysis

Logistic regression was performed in order to analyze the associations between dietary intake of vitamin D, omega-3 FA, folate and EULAR response (**Paper I**) as well as associations between dietary intake of omega-3 FA, omega-6 FA, omega-6 to 3 FA ratio and Refractory Pain (**Paper IV**).

Dietary intake of vitamin D, omega-3 FA and folate were divided into quartiles in **Paper I** and dietary intake of omega 3 FA, omega 6 FA and omega 6 to 3 FA ratio were divided into tertiles in **Paper IV**. Quartile and tertile divisions were based on the intake of the total EIRA sample, including matched controls; first quartile or tertile was defined as referent group.

All the analyses were adjusted for potential confounders such as age, gender, smoking, total energy intake, dietary supplementation, BMI, education and physical activity. Age was categorized into eleven age groups of five-year range each, BMI into below 25 and above 25

kg/m², education into high school degree and university degree, physical activity level into sedentary to moderate occasional physical activity and moderate regular physical activity to regular exercise, total energy intake into tertiles (**Paper IV**) and supplementation into yes and no. Smoking adjustments were based on pack year(s).

In **Paper IV**, additional logistic regression analyses were performed in order to study the association between dietary intake of omega-3 FA, omega-6 FA, omega-6 to 3 FA ratio and inflammatory markers such as CRP (above 10 mg/L), ESR (above 13 mm (>median)), inflammatory pain (VAS pain above 40 mm and CRP above 10 mg/L), SJC (more than one swollen joint (median)), and DAS28 (above 3.2). All the analyses were adjusted for age, gender, smoking pack year(s), total energy intake and omega-3 FA/fish oil supplementation.

4.1.2 SLEVIC

4.1.2.1 Outline

SLEVIC is an ongoing case-control study that investigates clinical aspects of SLE and is based at Karolinska University Hospital Huddinge, Stockholm, Sweden. SLEVIC collects data of patients with established SLE, aged 18-70 years, fulfilling the 1982 revised ACR criteria for SLE as well as randomly selected healthy controls matched by age and gender from Huddinge area. SLEVIC initiated in in 2006 and the inclusion period was from August 2006 to December 2007. All participants were asked to complete a questionnaire regarding life style factors (including a FFQ), health aspects and demographic data at inclusion/baseline. In addition, participants gave blood samples and were also examined for further laboratory investigations. A seven year follow-up was completed in 2014, however, a FFQ was not included in the follow-up.

4.1.2.2 Dietary assessment

Participants of SLEVIC were asked to complete a FFQ at baseline. This self-reported FFQ involved frequency of intake of 88 food items and beverages as well as dietary supplementation during the previous year from inclusion. Completed FFQs were evaluated and an estimation of daily mean intake of several nutrients was calculated (µg per day, mg per day, g per day). The nutrient calculations were performed using nutrient composition values from the Swedish National Food Administration data [282]. The nutrient intake was computed by multiplying the frequency of consumption of each food item by the nutrient content of specified portions. The reliability of reported dietary intake of PUFAs from the FFQ was verified by the significant

correlation with PUFAs found in the subcutaneous fat. Dietary supplementation was not taken into account in the calculation of estimated nutrient intake.

Dietary intake of all the assessed nutrients was used in **Paper II**, and dietary intake of only micronutrient (vitamin and mineral) intake was used in **Paper V**.

4.1.2.3 Additional assessment

Disease activity for SLE was measured with SLEDAI and SLAM, in order to distinguish between low and moderate/high disease activity, a cut-off score at 6 of both SLEDAI and SLAM was set [283]. Organ damage was assessed using SLICC. Laboratory variables, such as CRP, LDL and glucose levels were measured from overnight fasting venous blood samples, taken at inclusion. Additional data of demographic lifestyle factors (i.e. age, BMI, smoking) and treatment history were obtained through SLEVIC database and medical records, respectively.

4.1.2.4 Outcome assessment

In **Paper II**, GC treatment was used as a proxy of the disease activity of SLE. In order to assess clinical outcome of GC treatment, data on GC use and dose levels were extracted from medical records at three time points; at inclusion, and at one year before and after inclusion. GC use over time was categorized into four categories; "none", "discontinued", "started" and "continued". Dose changes over time were categorized into "decreased", "unchanged" and "increased". GC treatment and higher dose levels (above 5.0 mg per day and 7.5 mg per day, respectively) were considered as more active SLE; unchanged or increased GC doses were considered as unfavorable outcomes.

Carotid plaque among SLEVIC participants were examined at inclusion, the methodology of plaque assessment has been reported previously. [284]. The right and left carotid arteries were examined with B-mode ultrasound and a duplex scanner. The carotid arteries were carefully examined in regards to wall changes and carotid plaque was defined as an IMT of greater than 1 mm. Plaque was screened in the common, internal and external carotid arteries and was scored based on the absence or presence of plaque. Plaque morphology in terms of echogenicity was assessed and graded into echolucent (grade 1), predominantly echolucent (grade 2), predominantly echogenic (grade 3), and echogenic (grade 4) [285]. Echolucency was defined with the arterial lumen as reference and echogenicity with the far wall adventitia as reference. **Paper V** compared, in regards to micronutrient intake, participants with and without atherosclerotic plaque (grade 1-4) and echolucent plaque (grade 1), respectively.

4.1.2.5 Study participants

SLEVIC provided data on 114 SLE patients, fulfilling the 1982 revised ACR criteria for SLE and 112 matched controls. The inclusion period for **Paper II** and **Paper V** was during August 2006 to December 2007. An overview of the participant selection is presented in figure 6.

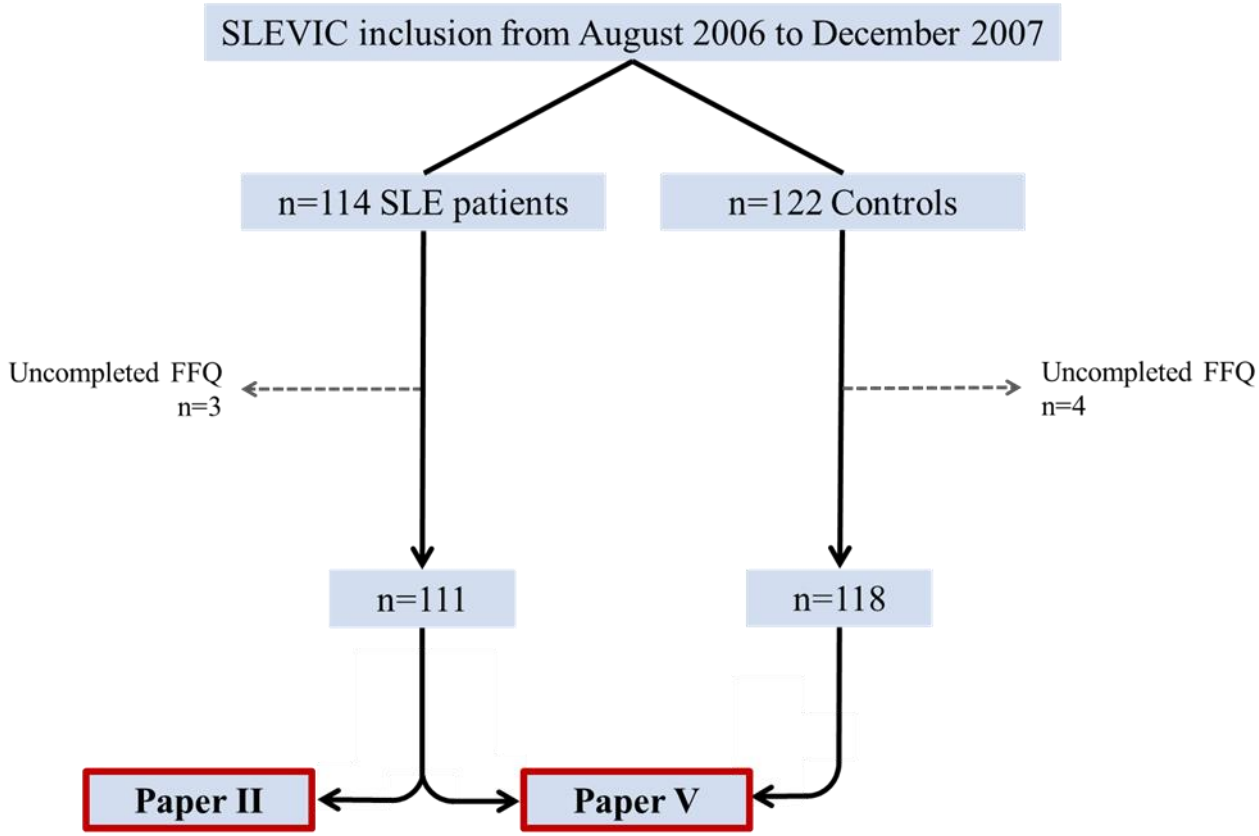

Figure 6. Overview of participant selection in **Paper II** and **Paper V**, respectively.

4.1.2.6 Statistical analysis

Logistic regression was the method used in order to analyze the association between dietary intake and GC treatment in SLE patients (**Paper II**) as well as association between dietary micronutrient intake and plaque in SLE patients and controls (**Paper V**).

In **Paper II**, Dietary data was linked with data on GC treatment during a two-year period (one year preceding and one year following the inclusion). Associations between dietary intake and

GC treatment in SLE patients were analyzed with logistic regression. The outcome of GC treatment was a) GC use (no or yes), b) GC dose change (decreased, unchanged, increased) and c) GC dose levels (above 5.0 and 7.5 mg per day). All nutrient variables were dichotomized into lower and higher intake with a cut-off at median levels, with an intake below median as the referent group. All the analyses were adjusted for age (continuous) and gender.

In **Paper V**, data on dietary micronutrient intake were linked with data on SLAM, SLEVIC and carotid plaque. Intake of 22 dietary micronutrients was compared between SLE patients and controls. Independent samples t-tests were performed for normally distributed variables and Mann-Whitney U test for skewed variables (skewness ±1.00). Associations between micronutrient intake and a) SLE (SLE or control), b) SLE activity (SLEDAI or SLAM above 6) and c) atherosclerotic (including echolucent) plaque (no or yes) were analyzed with logistic regression. Micronutrient variables were divided into tertiles based on the total intake from both SLE patients and controls, associations compared lower intake (1st tertile) with higher intake (3rd tertile). All analyses were adjusted for potential confounders such as age (continuous), LDL (continuous), glucose (continuous) and hypertension (yes or no).

4.1.3 SMC

4.1.3.1 Outline

The SMC is a population-based prospective cohort that includes women from Västmanland and Uppsala Counties in central Sweden and was established in 1987. The inclusion period was from March 1987 to December 1990 and included 90 303 women who were born in 1914 to 1948. These women were asked to complete questionnaires regarding life style factors (including a FFQ), health aspects, and demographic data. The response rate was 74% (n=66 651). Two follow-up questionnaires were sent out in 1997 and 2009 to those women who were still alive, still lived within the same counties and still fulfilled the inclusion criteria at these time points. The response rates were 70% (n=39 227 of 56 030 eligible women) in 1997 and 84% (n=25 332 of 30 134 eligible women) in 2009. The FFQs from 1997 and 2009 were most similar to each other for comparison purposes. SMC is based at IMM, Karolinska Insitutet Solna, Stockholm, Sweden.

The SMC was linked to both the SRQ as well as the Patient Register of the Swedish National Board of Health and Welfare in order to track women who were diagnosed with RA (incident patients) between 1997 and 2009. The Patient Register includes the Outpatient Register, that contains information on all the outpatient specialist visits from 2001, including day-surgery

and psychiatric visits from both public and private caregivers, and the Inpatient Register, that contains information on all hospitalizations from 1987.

4.1.3.2 Dietary assessment

The FFQs distributed in 1997 and 2009 included questions regarding the participants' frequency intake of several food items and beverages during the previous year from follow-up dates. The FFQ included 96 and 132 food items in 1997 and 2009, respectively. Frequency food intake ranged over eight categories from *Never* to *≥3 times per day*. Portion size (i.e. small, median, large) or quantity (i.e. slice, cup, glass, deciliter) of frequently consumed food items in Sweden was collected. Missing data and/or partial non-response for food items were coded as zero intake [286]. This project focused only on the dietary variables (food items) that were included in both FFQs in order to compare the dietary intake at the two time points; 82 common food items were identified.

The FFQs have previously been examined for their reproducibility and validity of reported food items and dietary supplementation and estimated nutrient intake through biomarkers, 24-hour recall interviews and/or national dietary records [287].

4.1.3.3 Study participants

Paper III included initially 21 840 women who had completed FFQ in 1997 and/or 2009. During the period from 1997 to 2009, 213 incident RA patients were identified. Women with uncompleted FFQ from 1997 and/or 2009 were excluded (n=82). After exclusion, there remained 191 women with RA and 21 567 women without RA in the study period. An overview of the participant selection are presented in figure 7.

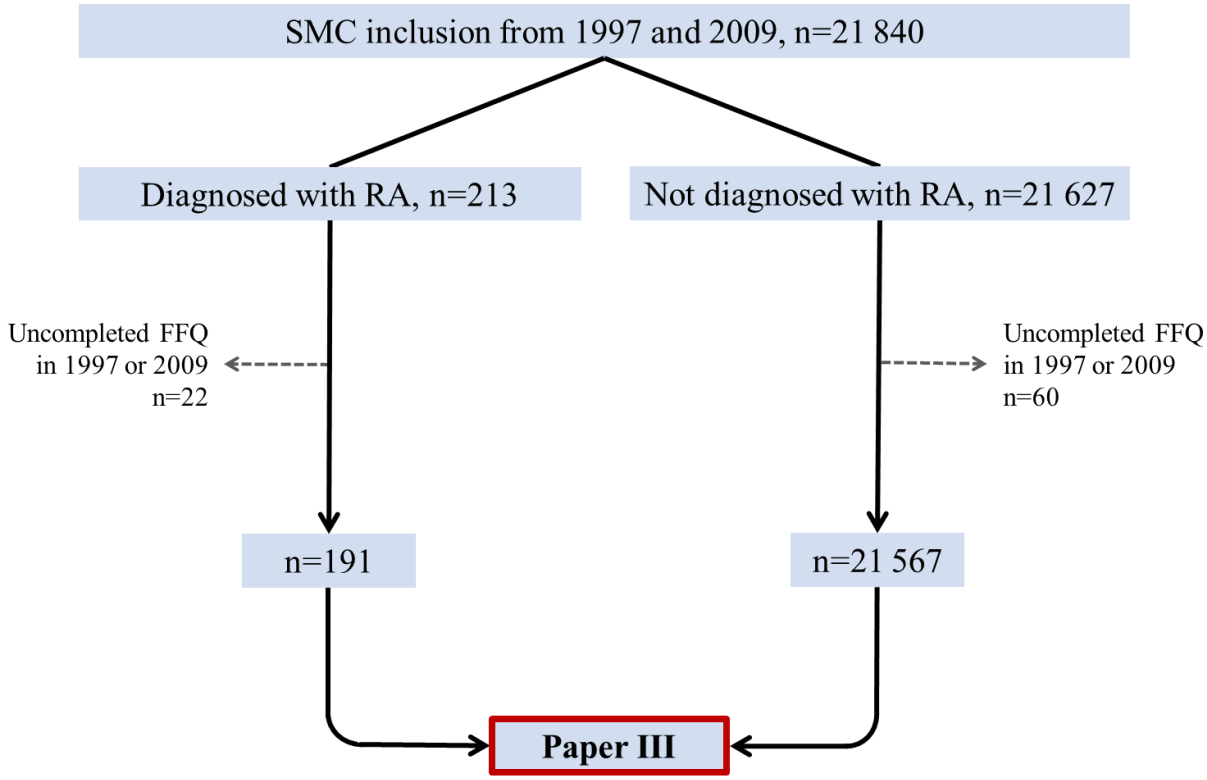

Figure 7. Overview of participant selection in **Paper III**.

4.1.3.4 Statistical analysis

Dietary changes after RA diagnosis were analyzed based on the food frequency intake in 1997 and 2009 with two approaches; linear mixed models and hierarchical cluster analysis.

Linear mixed models for repeated measures were used in order to compare the mean intake per week of the 82 common food items in FFQ from 1997 and 2009. Separate models were built for each outcome of interest (food item). Each food item was included as the dependent variable in the model, with 1997 and 2009 as fixed effects. These analyses were performed in both women with and without RA, separately. In addition, linear mixed models were performed in order to investigate if the changes in food intake from 1997 to 2009 differed between women with and without RA. All linear mixed models were adjusted for age, smoking, BMI and alcohol intake in 1997. Age (years), BMI (kg/m^2) and alcohol intake (g per day) were modeled as quartiles, while smoking status was categorized into never, former and current smoker.

Hierarchical cluster analysis using Ward's method was performed in order to identify subgroups of similar dietary patterns based on the difference of intake of all food items between 1997 and 2009 of both women with and without RA. For this analysis, we constructed a nested cases-control study using three controls of women without RA for each RA patient (n=573). The controls were randomly selected and matched by age, BMI and smoking.

4.1.4 SRQ

SRQ was founded in 1996 by The Swedish Society for Rheumatology (Swedish: Svensk Reumatologisk Förening (SRF)). It is a large nationwide ongoing self-reported registry of rheumatic patients. The goal of SRQ is to support patients and physicians to continuously improve care and patient outcomes for patients with rheumatic diseases. There are approximately 57,000 patients who are included in the SRQ. SRQ provides data on clinical outcomes of RA such as DAS28 and VAS (for pain) as well as health assessment questionnaire (HAQ), CRP, ESR, patients' and physician's global assessment, medical history etc.

Both EIRA and SMC has been linked with SRQ. Clinical data of RA patients used in **Paper I**, **Paper III** and **Paper IV** were obtained from the SRQ.

5 RESULTS

5.1 PAPER I: VITAMIN D, OMEGA-3 FA, FOLATE AND TREATMENT RESULTS IN RA

5.1.1 Characteristics of study participants

This study included 727 patients with early RA (symptom duration of less than one year) from EIRA, part II. Baseline characteristics of the study participants are presented in table 8. The proportion of current smokers was 31.9% and the proportion of patients who had obtained university degree and who had performed at least moderate regular physical activity or more during the previous year from baseline was 25.2% and 30.7%, respectively.

Table 8. Baseline characteristics.

	N=727
Female, %	72.6
Age (years), mean ± SD	52.5 ± 13.1
BMI, (kg/m²), mean ± SD	25.7 ± 4.6
Symptom duration (days), mean ± SD	302.3 ± 419.1
RF, positive, %	51.4
ACPA positive, %	66.2
DAS28, mean ± SD	5.2 ± 1.3
HAQ, mean ± SD	1.0 ± 0.6
CRP (mg/L), mean ± SD	22.6 ± 29.8
Pain (VAS 0-100 mm), mean ± SD	53.5 ± 24.7
Patients' global assessment (VAS 0-100 mm), mean ± SD	51.0 ± 24.4
Physicians' global assessment (5-point scale), mean ± SD	2.2 ± 0.7
SJC, mean ± SD	9.2 ± 5.4
TJC, mean ± SD	8.2 ± 5.9

ACPA, anti–citrullinated protein antibody; BMI, body mass index; CRP, C-reactive protein; DAS28, 28-joint disease activity score; HAQ, health assessment questionnaire; RF, rheumatoid factor; SD, standard deviation; SJC, swollen joint count; TJC, tender joint count; VAS, visual analogue scale

After three months of DMARD treatment, 399 patients (54.9%) had non- to moderate EULAR response and 328 patients (45.1%) had good EULAR response. Good responders, in comparison to non- to moderate responders, had significantly lower BMI (25.1 ± 4.4 versus 26.2 ± 4.8 kg/m2, p=0.005) and lower baseline TJC (7.5 ± 6.3 versus 8.8 ± 6.3, p=0.019). The

remaining baseline characteristics as well as smoking status, university degree and physical activity did not differ significantly between the different EULAR response groups.

5.1.2 Treatment use

The majority of the patients (89.9%) were initially treated with MTX monotherapy, followed by SSZ monotherapy and triple therapy (MTX, SSZ and HCQ). More than half of the patients (56.9%) were treated with DMARD(s) combined with GC. A similar pattern of treatment use was seen at the three month follow-up. (Table 9) Triple therapy at baseline was more common in good responders (3.7% versus 1.7%, p=0.046) and combined therapy of MTX and SSZ at three month follow-up was more common in non- to moderate responders (4.3% versus 1.0%, p=0.009).

Table 9. Treatment use at baseline and at three month follow-up.

	Baseline		**3 months**	
Treatment	**n (%)**	**GC use n (%)**	**n (%)**	**GC use n (%)**
MTX	653 (89.9)	373 (90.1)	579 (79.6)	351 (85.8)
SSZ	43 (5.9)	18 (4.3)	31 (4.3)	18 (4.4)
MTX + SSZ + HCQ	17 (2.3)	15 (3.6)	31 (4.3)	18 (4.4)
HCQ	8 (1.1)	3 (0.7)	9 (1.2)	5 (1.2)
LFM	2 (0.3))	1 (0.2)	0 (0)	0 (0)
AZA	1 (0.1)	1 (0.2)	0 (0)	0 (0)
MTX + SSZ	1 (0.1)	1 (0.2)	19 (2.6)	9 (2.2)
HCQ + SSZ	1 (0.1)	0 (0)	2 (0.3)	2 (0.5)
HCQ + AZA	1 (0.1)	1 (0.2)	1 (0.1)	1 (0.2)
GC	414 (56.9)	-	432 (59.4)	-
Missing data	0 (0)	-	46 (6.3)	-

AZA, azathioprine; GC, glucocorticoids; HCQ, hydroxychloroquine; LFM, leuflunomide; MTX, methotrexate; SSZ, sulfasalazine.

5.1.3 Vitamin D, omega-3 FA, folate and treatment results

The mean intake of vitamin D, omega-3 FA, folate and supplement use in the whole study sample as well as in different EULAR response groups are presented in table 10. Mean omega-3 FA intake was significantly higher in good responders. No difference between responders and non-responders was seen for vitamin D and folate intake. Dietary supplement use did not differ significantly across EULAR response groups. The vitamin D intake was below the RDI

and the folate intake was borderline obtained to the RDI according to Nordic Nutrition Recommendations 2012 [288].

In addition, the mean intake of dietary folate in the whole study sample was significantly lower compared to 2 632 healthy controls (308.39 ± 107.09 versus 324.35 ± 121.71 µg per day, p=0.001). No significant differences in mean intake of vitamin D and omega-3 FA were seen between RA patients and controls.

Table 10. The mean intake of vitamin D, omega-3 FA, folate and dietary supplement use in the whole study sample as well as in different EULAR response groups.

		EULAR response at 3 months		
Nutrient intake [1]	**Total study sample (N=727)**	**Non-/Moderate (n=399)**	**Good (n=328)**	**p value**
Vitamin D				
Dietary intake, µg/day, mean ± SD	5.86 ± 2.30 [RDI: 10-20]	5.68 ± 2.15	6.07 ± 2.45	0.062
Supplementation, n (%)	57 (7.8)	29 (7.3)	28 (8.5)	0.580
Omega-3 FA				
Dietary intake, g/day, mean ± SD	0.68 ± 0.35 [RDI: ≥1 E%]	0.65 ± 0.30	0.71 ± 0.39	0.040
Supplementation, n (%)	142 (19.5)	85 (21.3)	57 (17.4)	0.222
Folate				
Dietary intake, µg/day, mean ± SD	308.39 ± 107.09 [RDI: 300-400]	308.28 ± 115.00	308.52 ± 101.57	0.417
Supplementation, n (%)	113 (15.5)	67 (16.8)	46 (14.0)	0.355

E%, energy percent; FA, fatty acids; RDI, Recommended daily intake (accoring to Nordic Nutrition Recommendations 2012 [288])

[1] Reported nutrient intake and supplementation during the previous year from baseline.

The recommendations are age and gender specific. Women and men ≥75 years are recommended a daily intake of 20 µg of vitamin D. Both women and men are recommended a daily intake of omega-3 FA that equals 1 energy percent (E%) of the total daily fat intake. Women of reproductive age are recommended a daily intake of 400 µg of folate.

Dietary vitamin D and omega-3 FA intake were associated with good EULAR response after adjustment for age, gender, smoking, total energy intake and supplementation. However, dietary folate intake did not significantly associate with EULAR response. (Table 11) Additional analysis showed that omega-6:3 FA did not associate with EULAR response (odds ratio (OR)= 0.72 [95% confidence interval (CI) 0.74 to 1.11]). Additional adjustments for BMI, education and physical activity did not change the ORs remarkedly. Similar results were seen

in subgroup of the 653 patients who were initially treated with MTX monotherapy at baseline after adjustment for age and gender (OR=1.63 [95% CI 1.03 to 2.57] for vitamin D, OR=1.65 [95% CI 1.05 to 2.60] for omega-3 and OR=1.20 [95% CI 0.76-1.89] for folate.

Table 11. Association between dietary intake of vitamin D, omega-3 FA, folate and EULAR response after three months, respectively.

Nutrient intake	N	OR (95 % CI) Age and gender adj	OR (95 % CI) Multivariable adj
Vitamin D	**727**		
1st quartile: ≤4.25 µg/day	182	1.00	1.00
2nd quartile: 4.26-5.42 µg/day	170	1.07 (0.70-1.64)	1.07 (0.69-1.65)
3rd quartile: 5.43-6.96 µg/day	184	1.15 (0.75-1.77)	1.25 (0.81-1.94)
4th quartile: >6.97 µg/day	191	1.75 (1.13-2.71)	1.71 (1.09-2.67)
p value		0.012	0.019
Omega-3 FA	**727**		
1st quartile: ≤0.45 g/day	180	1.00	1.00
2nd quartile: 0.46-0.62 g/day	192	1.25 (0.82-1.89)	1.27 (0.83-1.94)
3rd quartile: 0.63-0.83 g/day	183	1.35 (0.89-2.07)	1.37 (0.89-2.12)
4th quartile: >0.84 g/day	172	1.64 (1.07-2.53)	1.60 (1.03-2.48)
p value		0.024	0.038
Folate	**727**		
1st quartile: ≤244.88 µg/day	201	1.00	1.00
2nd quartile: 244.89-296.86 µg/day	182	1.32 (0.88-1.99)	1.36 (0.89-2.06)
3rd quartile: 296.87-365.70 µg/day	193	1.59 (1.07-2.38)	1.54 (1.02-2.33)
4th quartile: >365.71 µg/day	151	1.14 (0.74-1.75)	1.09 (0.70-1.70)
p value		0.557	0.712

CI, confidence interval; FA, fatty acids; OR, odds ratio.

Multivariable adjustment for age (eleven groups of five year range each), gender, smoking pack year(s), total energy intake (quartiles) and supplementation (vitamin D, omega-3 FA/fish oil and folic acid).

p value: Comparison between 4th and 1st quartiles.

5.2 PAPER II: DIET AND GC TREATMENT IN SLE

5.2.1 Characteristics of study participants

This study included 111 SLE patients from SLEVIC. Patient characteristics are presented in table 12.

Table 12. Patient characteristics at inclusion.

	N=111
Age (years), mean ± SD	48.0 ± 13.2
Female, n (%)	98 (88.3)
BMI (kg/m^2), mean ± SD	24.9 ± 4.5
Current smokers, n (%)	15 (13.5)
CRP (mg/L), mean ± SD	4.6 ± 6.6
ESR (mm), mean ± SD	23.2 ± 17.3
Glucose (mmol/L), mean ± SD	4.6 ± 0.9
SLAM, median (IQR)	6 (4-9)
SLEDAI, median (IQR)	2 (0-6)
SLICC-DI, median (IQR)	1 (0-3)

BMI, body mass index; CRP, C-reactive protein; ESR; erythrocyte sedimentation rate; IQR, interquartile range; SD, standard deviation; SLAM, systemic lupus activity measure; SLEDAI; SLE disease activity index; SLICC-DI, systemic lupus international collaboration clinics damage index.

5.2.2 Treatment use

During a two-year period (one year prior to and one year after inclusion), the proportion of patients who were treated with GC ranged between 56.8 to 59.5%. In addition to GC, the most common treatments were HCQ (40.5 to 46.8%) and AZA (18.9 to 25.2%). (Table 13) The majority of the patients with GC at inclusion combined their treatment with supplementation of calcium and vitamin D (66.7%), calcium (24.2%) or vitamin D (6.1%).

Table 13. Treatment use and doses at inclusion and one year before and after inclusion.

	-1 year		Inclusion		+1 year	
Treatment	**n (%)**	**Mean ± SD (mg/day)**	**n (%)**	**Mean ± SD (mg/day)**	**n (%)**	**Mean ± SD (mg/day)**
GC	63 (56.8)	5.9 ± 2.5	66 (59.5)	6.3 ± 3.9	64 (57.7)	7.7 ± 7.8
HCQ	46 (41.4)	251.0 ± 91.2	52 (46.8)	232.9 ± 81.1	45 (40.5)	247.2 ± 86.4
AZA	28 (25.2)	115.4 ± 41.2	21 (18.9)	104.0 ± 40.1	23 (20.7)	103.3 ± 33.1
MTX	8 (7.2)	13.1 ± 6.6	9 (8.1)	15.3 ± 4.8	7 (6.3)	14.7 ± 6.2
MMF	8 (7.2)	1406.3 ± 581.5	8 (7.2)	1068.8 ± 628.5	6 (5.4)	1041.7 ± 510.3
CyA	6 (5.4)	159.2 ± 34.7	6 (5.4)	176.7 ± 97.3	6 (5.4)	133.3 ± 40.8
CYC	0 (0)	-	1 (0.9)	700 [1]	0 (0)	-
GC pulse	0 (0)	-	0 (0)	-	0 (0)	-

AZA, azathioprine; CyA, cyclosporine; CYC, cyclophosphamide; SD, standard deviation; GC, glucocorticoids; HCQ, hydroxychloroquine; MMF, mycophenolate mofetil; MTX, methotreaxate.

[1] mg/month during 6 months

During the two-year period, 23.4% did not use GC, 4.5% discontinued their GC treatment, 5.4% started with GC treatment and 48.6% had continuous GC treatment. 18.0% of the patients had missing data. (Figure 8).

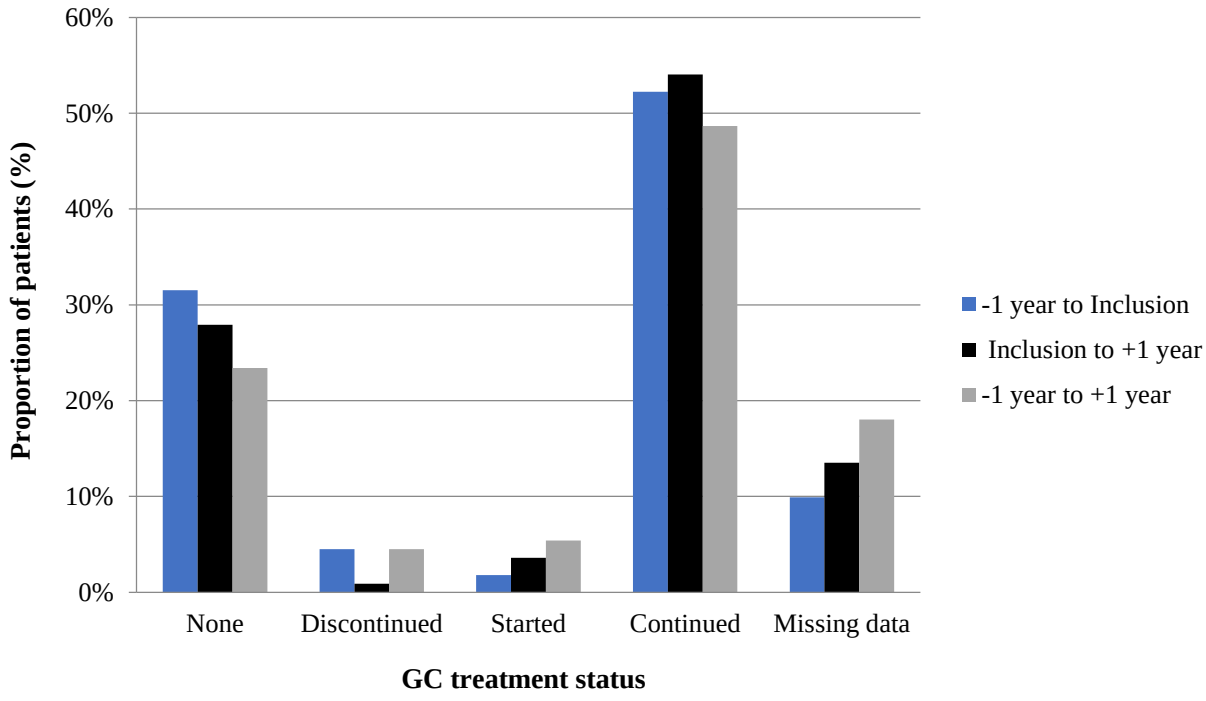

Figure 8. The distribution of GC treatment during one year before and after inclusion, and over the whole two-year period.

Of the 54 patients who had continued GC treatment over the two-year period, 31.5% had decreased dose, 35.2% had unchanged dose and 33.3% had increased dose. Changes in GC dose over the two-year period are presented in Table 14.

Table 14. GC dose change (decreased/unchanged/increased) over the two-year period.

	-1 year to Inclusion (n=58)		Inclusion to +1 year (n=60)		-1 year to +1 year (n=54)	
GC dose change	**n (%)**	**Mean ± SD (mg/day)**	**n (%)**	**Mean ± SD (mg/day)**	**n (%)**	**Mean ± SD (mg/day)**
Decreased	20 (34.5)	↘3.12 ± 1.30	14 (23.3)	↘4.51 ± 4.63	17 (31.5)	↘2.76 ± 1.9
Unchanged	24 (41.4)	-	25 (41.7)	-	19 (35.2)	-
Increased	14 (24.1)	↗4.76 ± 2.91	21 (35.0)	↗6.65 ±12.41	18 (33.3)	↗7.64 ±12.45

GC, glucocorticoids; SD, standard deviation.

5.2.3 Diet and GC treatment

Association between dietary nutrient intake and GC use is presented in Table 15. Vitamin D was associated with GC treatment (OR 2.70 to 2.85, [95% CI 1.00 to 8.11]) whereas alcohol was inversely associated with GC treatment (OR 0.28 to 0.39, [95% CI 0.10 to 98]).

Table 15. Association between dietary nutrient intake and GC use between -1 year to inclusion, inclusion to +1 year and -1 year to +1 year.

Time period	Nutrient [1]	OR [2]	95% CI	p value
-1 year to Inclusion	Alcohol (g)	0.39	0.16-0.98	0.045
Inclusion to +1 year	Alcohol (g)	0.31	0.12-0.79	0.015
	Vitamin D (µg) [3]	2.70	1.01-7.18	0.046
-1 year to +1 year	Alcohol (g)	0.28	0.10-0.79	0.016
	Vitamin D (µg) [3]	2.85	1.00-8.11	0.050

[1] All nutrients are dichotomized into lower (<median) and higher (>median) intake. Low intake = referent group.

[2] Odds ratio adjusted for age and gender.

[3] Not significant after adjusting for calcium/vitamin D supplementation.

Association between dietary nutrient intake and unchanged/increased GC dose is presented in Table 16. Beta-carotene, linoleic acid (fatty acid C18:2) and vitamin B_6 were inversely

associated with unchanged/increased GC dose, whereas vitamin B_{12} and calcium were associated with unchanged/increased GC dose. No associations were found between omega-3 FA, the omega-6 to -3 FA ratio and unchanged/increased GC dose.

Table 16. Association between dietary nutrient intake and GC dose change (decreased vs. unchanged/increased) between -1 year to inclusion, inclusion to +1 year and -1 year to +1 year.

Time period	Nutrient [1]	OR [2]	95% CI	p value
-1 year to Inclusion	Beta-Carotene (µg)	0.29	0.10-0.88	0.029
Inclusion to +1 year	Vitamin B_{12} (µg)	3.72	1.08-12.84	0.038
-1 year to +1 year	Linoleic acid (g)	0.30	0.10-0.90	0.031
	Vitamin B_6 (µg)	0.29	0.10-0.87	0.027
	Calcium (mg) [3]	5.36	1.64-17.52	0.005
	Vitamin B_{12} (µg)	3.20	1.08-9.43	0.035

[1] All nutrients are dichotomized into lower (<median) and higher (>median) intake. Low intake = referent group.

[2] Odds ratio adjusted for age and gender.

[3] After adjusting for calcium/vitamin D supplementation: OR=5.60 [95% CI 1.67-18.76]

The total energy intake was associated with GC dose higher than 5.0 mg per day and 7.5 mg per day, explaining an association between 35 nutrients and higher GC dose levels (OR 2.98-23.82 [95% CI 1.01-203.88]).

5.3 PAPER III: DIETARY CHANGES AFTER RA DIAGNOSIS

5.3.1 Characteristics of study participants

This study included 191 women who had been diagnosed with RA between 1997 and 2009 and 21 840 women who had not been diagnosed with RA. Baseline characteristics of the study participants are presented in table 17. There were no major differences between women with and without RA, except for smoking that was more common among women with RA.

Table 17. Baseline characteristics between women with and without RA diagnosis between 1997 and 2009.

Characteristics (1997)	**RA (n=191)**	**No RA (n=21 567)**	**p value**
Age (years), mean ± SD	59 ± 7	59 ± 8	N.S.
BMI (kg/m^2), mean ± SD	24.5 ± 3.6	25.0 ± 3.8	N.S.
Smoking, %			<0.001
• Never	33.9	52.7	
• Former	31.7	25.6	
• Current	34.4	21.8	
Education, %			N.S.
• Less than high school	33.0	34.7	
• High school	46.1	41.7	
• University	20.9	23.6	

SD, standard deviation; N.S., Not significant

5.3.2 Dietary intake

The majority of the 82 food items did not differ significantly in intake between women with and without RA in 1997 and in 2009 (figure 9).

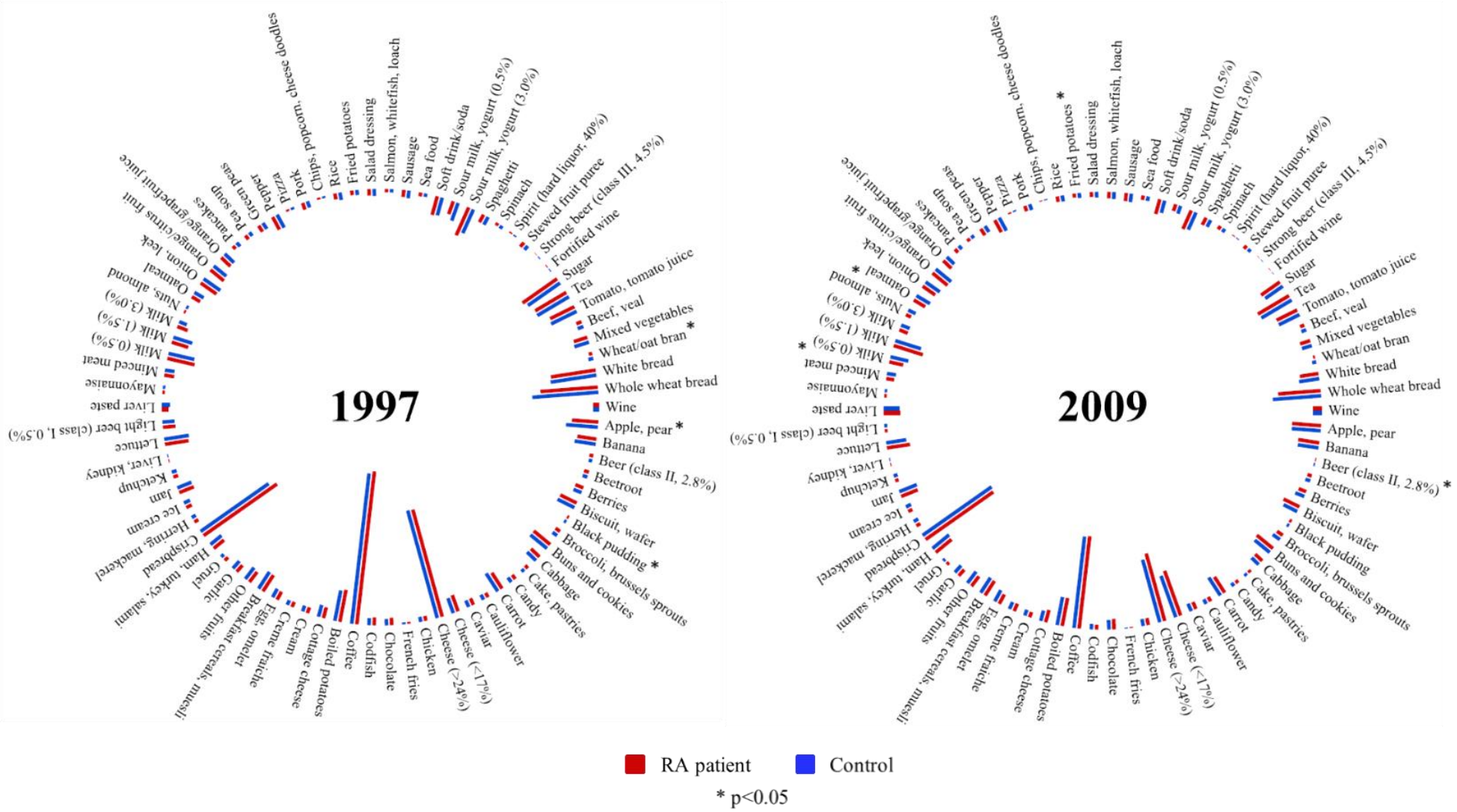

Figure 9. Dietary intake between women with and without RA in 1997 and 2009, respectively.

Women with RA had statistically significantly lower intake of apple/pear, black pudding and wheat/oat bran in 1997, and beer (2.8% alcohol), fried potatoes, low-fat milk and oatmeal in 2009, compared to women without RA diagnosis (table 18). Mean intakes of all the food items between women with and without RA at the two time points are presented in Appendix 1:2 of **Paper III** at the end of this thesis.

Table 18. Mean intake among women with (n=191) and without RA (n=21,567) in 1997 and 2009.

	Food item	Intake (servings per week)		p value
		RA (mean ± SD)	No RA (mean ± SD)	
1997	Apple, pear	3.64 ± 0.51	4.41 ± 0.58	0.003
	Black pudding	0.28 ± 0.06	0.35 ± 0.10	0.027
	Wheat/oat bran	0.49 ± 0.21	0.70 ± 0.28	0.008
2009	Beer (class II, 2.8%)	0.14 ± 0.49	0.21 ± 0.56	0.049
	Fried potatoes	0.56 ± 0.84	0.70 ± 0.98	0.002
	Low-fat milk	1.96 ± 5.95	2.52 ± 6.02	0.045
	Oatmeal	1.82 ± 2.66	2.17 ± 2.80	0.049

SD, standard deviation.

5.3.3 Changes in food intake between 1997 and 2009

Results from mixed models showed that the food frequency intake of 44 (53.7%) food items changed significantly from 1997 to 2009 *among* women with RA and 82 (100%) food items *among* women without RA. The changes of each food item during the study period (decreased or increased intake) were found to be the same in the two groups.

Changes in dietary intake from 1997 to 2009 did not differ significantly *between* women with and without RA for 79 (96.3%) food items. The very few significant differences were intake of rice, wheat/oat bran and whole wheat bread. The increase in intake of these three food items was higher among women without RA than among women with RA. (Figure 10)

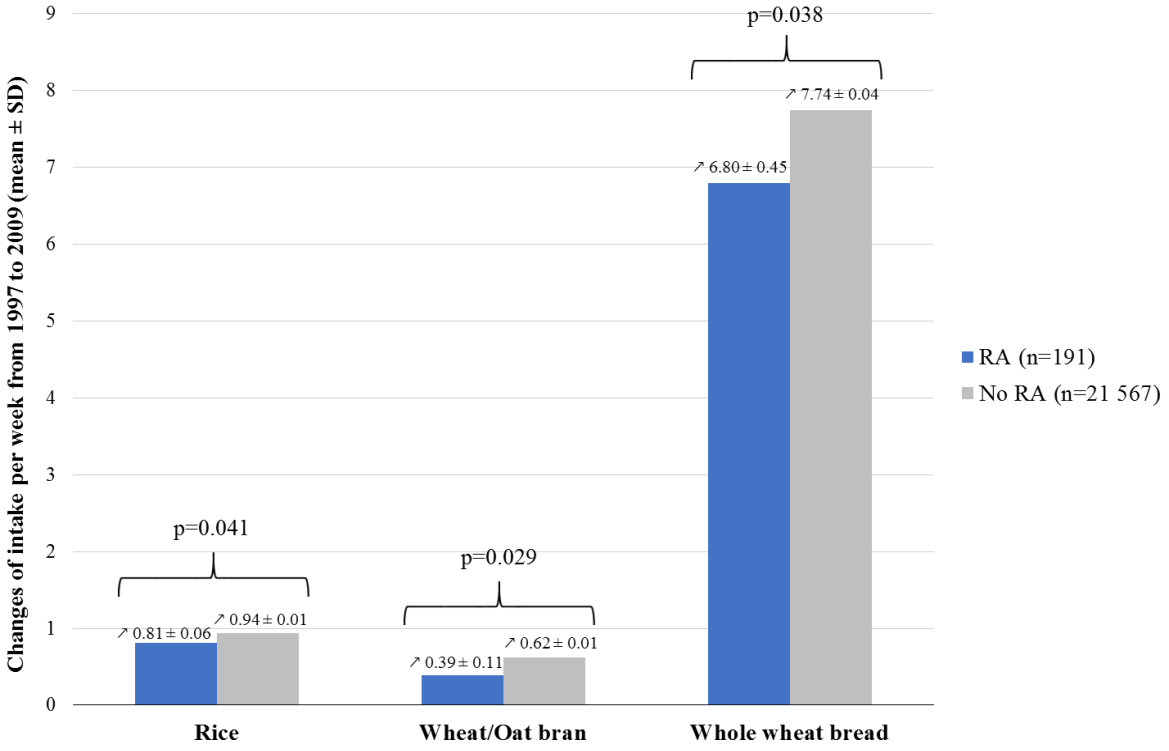

Figure 10. Multivariable adjusted changes in dietary intake (servings/week) from 1997 and 2009. Analyses were adjusted for age (quartiles), smoking (never, former, current), BMI (quartiles) and alcohol intake (quartiles) in 1997.

Based on the results presented in figure 10, further mixed model analyses comparing whole grains (crisp bread, whole wheat bread, oat meal, gruel, cereals/muesli) and refined grains (white bread/loaf, pasta, rice, pancakes, biscuit/wafer, buns/cakes) were performed in the same manner. The results showed no statistically significant differences regarding types of grains between women with and without RA. (Table 19)

Table 19. Multivariable adjusted changes in dietary intake (servings/week) from 1997 and 2009.

	Servings per week 1997→2009 (mean ± SD)		
Type of grains	**RA (n=191)**	**No RA (21 567)**	**p value**
Whole	↗23.73 ± 0.79	↗24.25 ± 0.08	0.515
Refined	↗12.2 ± 0.53	↗11.80 ± 0.05	0.519

SD, standard deviation

Adjustment for age (quartiles), smoking (never, former, current), BMI (quartiles) and alcohol intake (quartiles) in 1997.

5.3.4 Dietary patterns

The hierarchical cluster analysis did not identify any meaningful clusters based on the total intake of all food items. This did not allow further comparison of dietary pattern changes over time between RA patients and controls. (Figure 11)

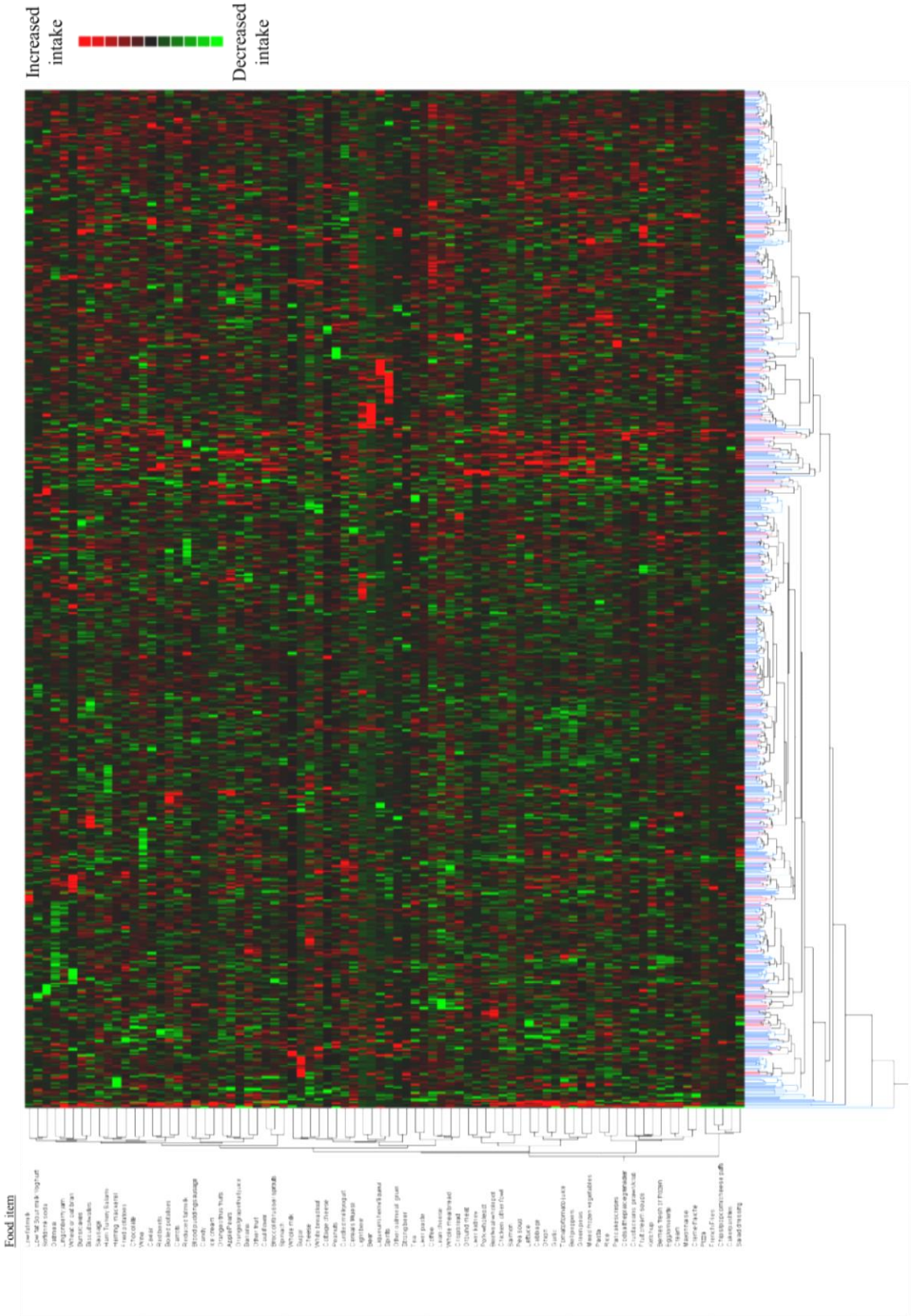

Figure 11. Dendrogram from hierarchical cluster analysis. The Y-axis shows clusters based on the changes in dietary intake of all the food items between 1997 and 2009, where increased to decreased intake is colored in a red-to-green scale. The X-axis demonstrates all the participants in each cluster, where RA patients and controls are colored in red and blue, respectively.

5.4 PAPER IV: PUFA AND PAIN IN RA

5.4.1 Characteristics of study participants

This study included 591 RA patients. Baseline characteristics of the total study sample as well as patients with and without Refractory Pain, separately, are presented in table 20. The proportion of smokers was 30.8%, with university degree 24.0%, and those who had performed at least moderate regular physical activity or more 31.6%. After three months of MTX treatment, 92 patients (15.6%) had Refractory Pain. Of these patients, 16.3% had good, 44.6% had moderate and 32.6% had no EULAR response (missing data: 6.5%).

Table 20. Baseline characteristics.

	Total study sample	**Refractory Pain after 3 months of MTX**		
	N=591	**No (n=499)**	**Yes (n=92)**	**p value [1]**
Female, %	70.6	70.9	68.5	N.S.
Age (years), mean ± SD	52.8 ± 13.0	53.0 ± 13.2	51.5 ± 11.5	N.S.
BMI, (kg/m²), mean ± SD	25.8 ± 4.7	25.6 ± 4.7	26.5 ± 4.5	N.S.
Smoking status: Never / Former / Current, %	33.0 / 35.9 / 30.8	34.1 / 36.9 / 28.7	27.2 / 30.4 / 42.4	0.034
Symptom duration (days), mean ± SD	289.7 ± 390.6	290.9 ± 401.7	283.2 ± 325.4	N.S.
RF, positive, %	65.3	65.3	65.2	N.S.
ACPA positive, %	67.9	68.3	65.2	N.S.
DAS28, mean ± SD	5.2 ± 1.3	5.1 ± 1.3	5.4 ± 1.4	N.S.
CRP (mg/L), mean ± SD	22.4 ± 27.2	23.2 ± 28.1	18.0 ± 21.7	N.S.
ESR (0-100 mm), mean ± SD	32.0 ± 21.7	32.4 ± 22.3	29.8 ± 18.4	N.S.
HAQ (VAS 0-100 mm), mean ± SD	1.1 ± 0.6	1.0 ± 0.6	1.3 ± 0.6	<0.001
Pain (VAS 0-100 mm), mean ± SD	53.9 ± 24.7	52.5 ± 24.9	60.1 ± 22.8	0.002
Physician's GA (5-point scale), mean ± SD	2.2 ± 0.7	2.2 ± 0.7	2.2 ± 0.7	N.S.
Patients' GA (0-100 mm), mean ± SD	50.5 ± 24.4	49.1 ± 24.7	57.6 ± 21.1	0.001
SJC, mean ± SD / median (IQR)	9.3 ± 5.5 / 9 (5-12)	9.2 ± 5.4 / 8 (5-12)	9.7 ± 5.9 / 9 (5-14)	N.S.
TJC, mean ± SD / median (IQR)	8.2 ± 5.9 / 7 (4-12)	7.9 ± 5.8 / 7 (3-12)	10.1 ± 6.3 / 9 (5-14)	0.002
MTX, %	100.0	100.0	100.0	N.S.
GC, %	59.1	59.9	54.3	N.S.
COX-1 inhibitor, %	52.6	52.7	52.2	N.S.
COX-2 inhibitor, %	1.7	1.6	2.2	N.S.
Omega-3 FA/fish oil supplementation, %	19.5	20.0	16.3	N.S.
Omega-3 FA intake (g/day), mean ± SD	0.69±0.37	0.71±0.38	0.60±0.30	0.004
Omega-6 FA intake (g/day), mean ± SD	7.60±2.33	7.57±37	7.81±2.08	N.S.
Omega-6:3 FA, mean ± SD	13.37±7.21	12.91±6.91	15.86±8.30	<0.001

ACPA, anti–citrullinated protein antibody; BMI, body mass index; COX, cyclooxygenase; CRP, C-reactive protein; DAS28, 28-joint disease activity score; ESR, erythrocyte segmentation rate; FA, fatty acid; GA, global assessment; GC, glucocorticoids; HAQ, health assessment questionnaire; IQR, inter quartile range; MTX, methotrexate; N.S., not significant; RF, rheumatoid factor; SJC, swollen joint count; SD, standard deviation; TJC, tender joint count.

[1] Comparison between with and without Refractory Pain.

5.4.2 PUFA intake

The mean intake of omega 3 FA, omega 6 FA and omega 6 to 3 FA for the whole study group was 0.69±0.37 g per day, 7.60±2.33 g per day and 13.37±7.21, respectively. The omega 3 FA/fish oil supplementation was used by 19.5% of the patients. Omega 3 FA intake was found to be lower in patients with Refractory Pain compared to those without Refractory Pain (0.60±0.30 vs. 0.71±0.38 g per day, p=0.004). The proportion of omega 3 FA/fish oil supplementation users did not differ significantly between patients with and without Refractory Pain. (Table 20).

5.4.3 Omega 3 FA, omega 6 FA, omega 6 to 3 FA and Remaining Pain

Omega 3 FA was inversely associated with Refractory Pain and omega 6 to 3 FA ratio was directly associated with Refractory Pain, after adjustment for age, gender, smoking, total energy intake and omega 3 FA/fish oil supplementation (OR=0.46 [95% CI 0.26 to 0.83] and OR=2.44 [95% 1.35 to 4.42], respectively). These associations were based on comparison between the 3rd and the 1st tertile. Additional adjustments for ACPA, BMI, education and physical activity did not change the ORs remarkably. Omega 6 FA alone did not significantly associate with Refractory Pain, however, a positive significant association was seen between 2nd versus the 1st tertile and Refractory Pain. (Table 21)

Table 21. Association between dietary intakes of omega-3, omega-6, omega-6:3 FA and refractory pain after three months of MTX treatment.

PUFA intake	N	OR_{crude} (95 % CI)	$OR_{adjusted}$ (95 % CI)
Omega-3 FA	**591**		
1st tertile: ≤0.48 g/day	206	1.00	1.00
2nd tertile: 0.49-0.77 g/day	186	0.67 (0.40-1.14)	0.66 (0.38 to 1.13)
3rd tertile: >0.78 g/day	199	0.44 (0.25-0.77)	0.46 (0.26 to 0.83)
p value		0.005	0.010
Omega-6 FA	**591**		
1st tertile: ≤6.00 g/day	192	1.00	1.00
2nd tertile: 6.01-8.19 g/day	196	1.98 (1.10-3.57)	1.85 (1.02 to 3.36)
3rd tertile: >8.20 g/day	203	1.77 (0.94-3.32)	1.61 (0.85 to 3.08)
p value		0.075	0.146
Omega-6:3 FA	**591**		
1st tertile: ≤9.74	202	1.00	1.00
2nd tertile: 9.75-14.19	191	1.49 (0.80-2.76)	1.37 (0.72 to 2.59)
3rd tertile: >14.20	198	2.63 (1.48-4.68)	2.44 (1.35 to 4.42)
p value		0.001	0.003

OR_{crude}: Adjustment for age and gender.

$OR_{adjusted}$: Adjustment for age (eleven groups of five year range each), gender, smoking pack year(s), total energy intake (tertiles) and omega-3 FA/fish oil supplementation.

p values: Comparison between 3rd and 1st tertiles.

No association was observed between omega 3 FA/fish oil supplementation and Refractory Pain after adjustment for age, gender, smoking, total energy intake and dietary intake of omega 3 FA (OR=0.84 [95% CI 0.46 to 1.55]).

5.4.4 Omega 3 FA, omega 6 FA, omega 6 to 3 FA and inflammatory markers

In order to test whether low inflammatory state contributed in a major way to the results presented in table 21, a sensitivity analysis was performed using only unbearable pain (VAS pain above 40 mm). In a similar way as for Refractory Pain, unbearable pain was statistically significantly associated with omega 3 FA (OR=0.54 [95 % CI 0.33-0.89]) and with omega 6 to 3 FA ratio (OR=1.86 [95% CI 1.14-3.05]). The association between unbearable pain and Omega 6 FA was non-significant (OR=1.39 [0.89-2.38]). Moreover, in multivariable adjusted analyses, dietary omega 3 FA, omega 6 FA and omega 6 to 3 FA ratio did significantly associate with inflammatory markers; neither with CRP, ESR, inflammatory pain, SJC nor with DAS28 after 3 months, respectively. (Table 22)

Table 22. Dietary intake of omega-3, omega-6, omega-6:3 FA and their association to inflammatory parameters (CRP, ESR, inflammatory pain, SJC and DAS28) after three months of MTX treatment.

	PUFA intake (g/day)	N	OR (95 % CI) [1]	p value
CRP	Omega-3	581	1.25 (0.69-2.29)	0.462
	Omega-6	581	0.84 (0.44-1.61)	0.605
	Omega-6:3	581	0.63 (0.34-1.16)	0.137
ESR	Omega-3	561	1.99 (0.64-1.51)	0.945
	Omega-6	561	0.95 (0.60-1.48)	0.806
	Omega-6:3	561	0.84 (0.55-1.28)	0.420
Inflammatory pain	Omega-3	581	1.27 (0.53-3.02)	0.566
	Omega-6	581	0.77 (0.31-1.94)	0.582
	Omega-6:3	581	0.59 (0.24-1.44)	0.214
SJC	Omega-3	579	0.92 (0.60-1.42)	0.715
	Omega-6	579	0.90 (0.55-1.43)	0.643
	Omega-6:3	579	0.92 (0.60-1.41)	0.714
DAS28	Omega-3	559	0.69 (0.45-1.06)	0.087
	Omega-6	559	1.11 (0.71-1.72)	0.659
	Omega-6:3	559	1.24 (0.82-1.88)	0.309

CRP, C-reactive protein; ESR, erythrocyte segmentation rate; SJC, swollen joint count; DAS28, 28-joint disease activity score.

[1]Adjustment for age, gender, smoking pack year(s), total energy intake and omega-3 FA/fish oil supplementation. Comparison between 3rd and 1st tertiles.

CRP >10.00 mg/L

ESR >13.00 mm (median)

Inflammatory pain: VAS pain >40 mm and CRP >10 mg/L

SJC >1 swollen joint (median)

DAS28 >3.2

In addition, there was no significant association observed between use of omega 3/fish oil supplements and any of the inflammatory markers, after adjustment for age, gender, smoking, total energy intake and dietary intake of omega 3 FA (OR=1.04 [95% CI 0.56 to 1.94] for CRP; OR=1.15 [95% CI 0.75 to 1.76] for ESR; OR=1.39 [95% CI 0.60 to 3.24] for inflammatory pain; OR=1.20 [95% CI 0.82 to 1.94] for SJC, OR=1.02 [95% CI 0.67 to 1.56] for DAS28).

5.5 PAPER V: MICRONUTRIENTS AND ATHEROSCLEROSIS IN SLE

5.5.1 Characteristics of study participants

This study included 111 SLE patients, with median disease duration of 9 years, and 118 matched controls. Characteristics of the study participants are presented in table 23. Fifty-three patients (47.7%) had SLAM above 6 and 23 patients (20.7%) had SLEDAI above 6. Atherosclerotic and ecolucent plaque on the left side were more common in SLE patients than controls (p=0.008 and 0.006, respectively).

Table 23. Characteristics of the study participants.

	Patients (N=111)	**Controls (N=118)**	**p value**
Age (years), mean ± SD	48.0± 13.2	49.5 ± 12.6	N.S.
Female, n (%)	98 (88.3)	105 (89.0)	N.S.
BMI (kg/m^2), mean ± SD	24.9 ± 4.5	25.4 ± 5.2	N.S.
Smokers, n (%)	15 (13.5)	19 (16.2)	N.S.
CRP (mg/L), mean ± SD	4.9 ± 6.6	2.1 ± 3.2	0.001
LDL (mmol/L), mean ± SD	2.5 ± 0.9	2.8 ± 0.8	0.031
Glucose (mmol/L), mean ± SD	4.6 ± 0.9	4.9 ± 0.9	≤0.001
Hypertension, (>140/90) n (%)	66 (59.5)	31 (26.3)	≤0.001
SLAM, median (IQR)	6 (4-9)	-	-
SLEDAI, median (IQR)	2 (0-6)	-	-
SLICC, median (IQR)	1 (0-3)	-	-
Atherosclerotic plaque (grade 1-4)			
- Left and/or right side, n (%)	48 (43.2.0)	36 (30.5)	0.046
- Left side, n (%)	39 (35.1)	23 (19.5)	0.008
Echolucent plaque (grade 1), n (%)			
- Left and/or right side, n (%)	33 (29.7)	23 (19.5)	N.S.
- Left side, n (%)	25 (22.5)	12 (10.2)	0.006

BMI, body mass index; CRP, C-reactive protein; IQR, interquartile range; LDL, low density lipoprotein; N.S., not significant; SLAM, systemic lupus activity measure; SLEDAI, systemic lupus erythematosus disease activity index; SLICC, systemic lupus international collaboration clinics damage index.

5.5.2 Micronutrient intake

Mean micronutrient intake did not differ significantly between patients and controls (table 24), and between SLE patients with lower and higher disease activity (SLAM, SLEDAI

below/above 6). However, higher phosphorus intake (>2 497.35 mg per day) was associated with SLEDAI above 6, after adjusting for age and LDL (OR=2.82 [96% CI 1.06 to 7.52]).

Table 24. Dietary micronutrient intake (mg per day, µg per day) in SLE patients and healthy controls.

Micronutrient	Patients (N=111) Mean ± SD	Controls (N=118) Mean ± SD	p value
Vitamin C (mg)	139.9 ± 91.1	145.4 ± 71.3	N.S. [1]
Vitamin B_6 (mg)	2.2 ± 0.8	2.2 ± 0.7	N.S. [1]
Vitamin E (mg)	8.2 ± 3.2	8.5 ± 2.9	N.S. [1]
Iron (mg)	13.3 ± 5.3	13.6 ± 5.3	N.S. [1]
Calcium (mg)	1279.7 ± 639.5	1253.2 ± 548.3	N.S. [1]
beta-Carotene (µg)	4197.0 ± 3001.4	4682.3 ± 3089.1	N.S. [1]
Magnesium (mg)	393.3 ± 144.0	403.4 ± 134.5	N.S. [1]
Retinol equivalent (µg)	1464.7 ± 727.8	1539.5 ± 697.1	N.S. [1]
Retinol (µg)	722.1 ± 415.7	704.5 ± 416.6	N.S. [1]
Riboflavin (mg)	2.0 ± 0.9	1.9 ± 0.7	N.S. [1]
Selenium (µg)	36.6 ± 17.1	36.2 ± 12.1	N.S. [1]
Thiamin (mg)	1.4 ± 0.5	1.3 ± 0.4	N.S. [2]
alpha-Tocopherol (mg)	8.0 ± 3.2	8.3 ± 2.9	N.S. [1]
Vitamin D (µg)	6.0 ± 3.1	5.8 ± 2.3	N.S. [1]
Niacin (mg)	17.1 ± 6.1	17.2 ± 5.2	N.S. [1]
Niacin equivalent (mg)	34.4 ± 12.1	34.8 ± 10.3	N.S. [1]
Vitamin B_{12} (µg)	6.0 ± 3.0	6.0 ± 2.7	N.S. [1]
Phosphorus (mg)	1721.2 ± 680.9	1715.1 ± 598.4	N.S. [2]
Sodium (mg)	3081.8 ± 1049.8	3036.2 ± 954.8	N.S. [2]
Potassium (mg)	3711.1 ± 1446.6	3716.1 ± 1141.4	N.S. [1]
Zinc (mg)	12.0 ± 4.3	12.4 ± 4.4	N.S. [1]
Folate (µg)	362.0 ± 205.5	375.7 ± 150.9	N.S. [1]

N.S., not significant; SD, standard deviation.

[1] Mann-Whitney U test

[2] Independent samples t-test

5.5.3 Micronutrients and plaque

5.5.3.1 In SLE patients

Some micronutrients were found to associate with presence of atherosclerotic plaque on the left side, after adjustment for age, LDL, glucose and hypertension. Patients with lower intake of riboflavin (≤1.43 mg per day) and phosphorus (≤1,356.03 mg per day) had higher odds of atherosclerotic plaque on the left side (OR=3.06 [95% CI 1.12 to 8.40] and OR=4.36 [95% CI

1.53 to 12.39], respectively), and patients with higher intake of selenium (>40.84 µg per day) and thiamin (>1.50 mg per day) had lower odds of atherosclerotic plaque on the left side (OR=0.28 [95% CI 0.09 to 0.89] and OR=0.26 [95% CI 0.08 to 0.80], respectively). (Table 25)

Table 25. Association between lower and higher micronutrient intake (mg per day, µg per day) and atherosclerotic plaque (grade 1-4) on the left side in SLE patients.

	Atherosclerotic plaque (left side)					
	Lower intake (≤1st tertile)			**Higher intake (>3rd tertile)**		
Micronutrient [1]	**OR [2]**	**95% CI**	**p value**	**OR [2]**	**95% CI**	**p value**
Vitamin C (mg)			N.S.			N.S.
Vitamin B_6 (mg)			N.S.			N.S.
Vitamin E (mg)			N.S.			N.S.
Iron (mg)			N.S.			N.S.
Calcium (mg)			N.S.			N.S.
beta-Carotene (µg)			N.S.			N.S.
Magnesium (mg)			N.S.			N.S.
Retinol equivalent (µg)			N.S.			N.S.
Retinol (µg)			N.S.			N.S.
Riboflavin (mg)	**3.06**	**1.12 to 8.40**	**0.030**			N.S.
Selenium (µg)			N.S.	**0.28**	**0.09 to 0.89**	**0.031**
Thiamin (mg)			N.S.	**0.26**	**0.08 to 0.80**	**0.019**
alpha-Tocopherol (mg)			N.S.			N.S.
Vitamin D (µg)			N.S.			N.S.
Niacin (mg)			N.S.			N.S.
Niacin equivalent (mg)			N.S.			N.S.
Vitamin B_{12} (µg)			N.S.			N.S.
Phosphorus (mg)	**4.36**	**1.53 to 12.39**	**0.006**			N.S.
Sodium (mg)			N.S.			N.S.
Potassium (mg)			N.S.			N.S.
Zinc (mg)			N.S.			N.S.
Folate (µg)			N.S.			N.S.

N.S., not significant; SD, standard deviation.

[1] All nutrients were dichotomized: Lower intake: ≤1st tertile and >1st tertile (>1st tertile = referent group). Higher intake: ≤3rd tertile and >3rd tertile (≤3rd tertile = referent group).

[2] Odds ratio adjusted for age (continuous), LDL (continuous), glucose (continuous) and hypertention (no/yes).

In addition, higher intake of thiamin had lower odds of echolucent plaque on the left side, after adjusting for age and LDL (OR=0.22 [95% CI 0.06 to 0.84]. Moreover, patients without any

atherosclerotic plaque on the left side had significantly higher daily intake of several micronutrients (vitamin C, Vitamin B_6, riboflavin, thiamin, niacin, phosphorus, sodium, and zinc) compared to patients with atherosclerotic plaque on the left side (results not shown).

Overall, no micronutrients were significantly associated with atherosclerotic plaque and echolucent plaque on the right side. Only lower intake of folate (≤255.21 µg per day) had decreased odds of bilateral echolucent plaque (OR=0.36 (95% CI 0.13 to 0.99).

5.5.3.2 In controls

Higher intake of beta-carotene (>4921.21 µg per day) and niacin equivalent (>37.58 mg per day) were inversely associated with atherosclerotic plaque on the right side, after adjusting for age and LDL (OR=0.33 [95% CI 0.11 to 0.96] and OR=0.23 [95% CI 0.07 to 077], respectively), but not on the left side. No associations were found between micronutrient intake and bilateral atherosclerotic and echolucent plaque, after adjusting for potential confounders.

6 DISCUSSION

This thesis has focused on potential associations between dietary factors and treatment outcome in patients with RA and SLE. Dietary aspects of rheumatic diseases have been highlighted in the media over the last decade. Several dietary studies have reported benefits for both RA and SLE patients. However, many of these studies have mainly focused on the dietary impact on the disease status, and the use of treatment has not always been considered. Thus, **Paper I**, **Paper II** and **Paper IV** have examined the link between diet and clinical outcomes in regards to anti-rheumatic treatment in RA and SLE. In addition, **Paper III** and **Paper V** have studied the long-term changes after RA diagnosis and the link between diet and atherosclerotic plaque in SLE, respectively. An overview of the main results from **Papers I-V** is presented in figure 12.

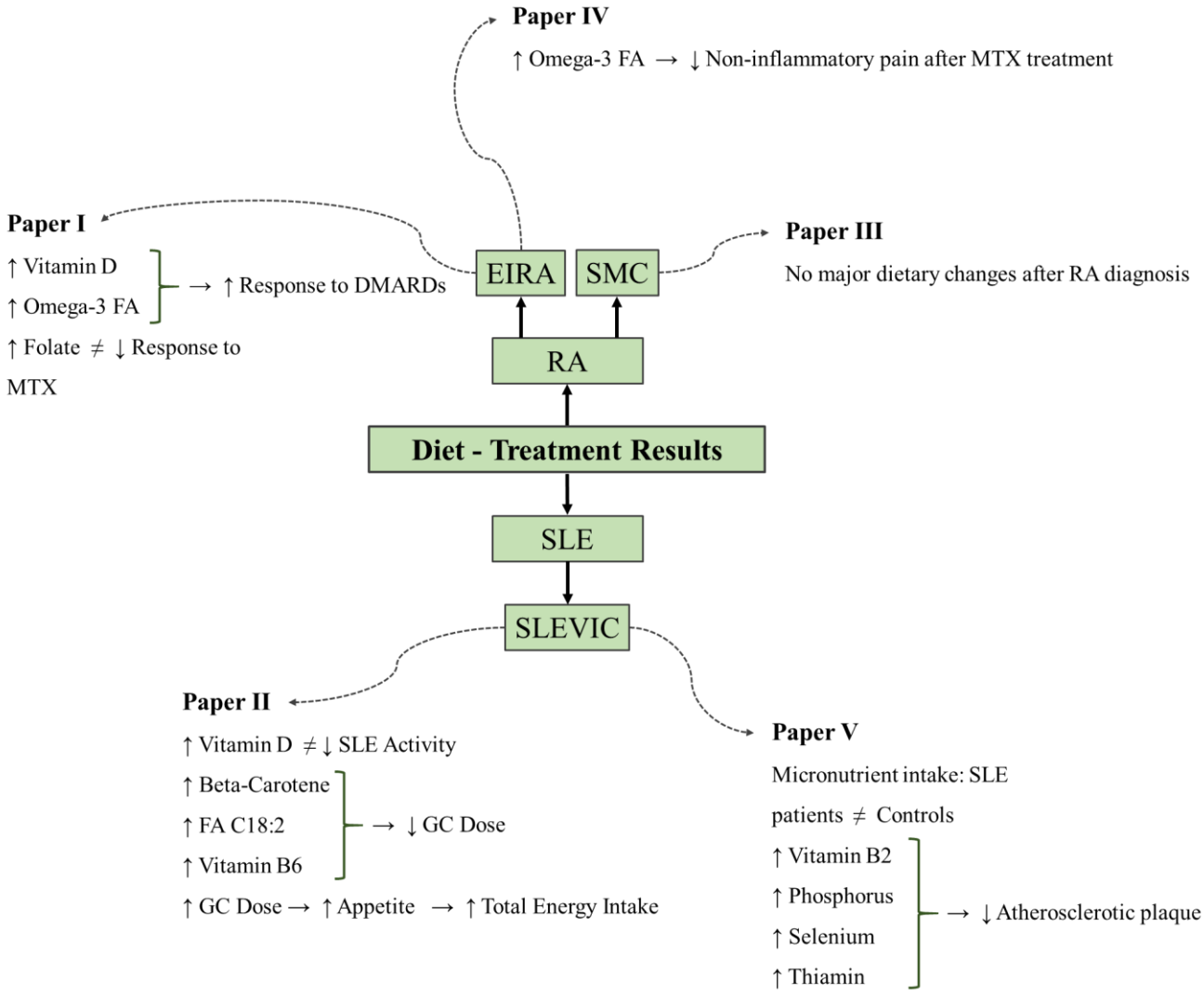

Figure 12. Overview of the main results of **Papers I-V**.

6.1 METHODOLOGICAL CONSIDERATIONS

6.1.1 Dietary assessments

6.1.1.1 FFQ

FFQs are very useful and inexpensive tools for estimating dietary intake in larger populations. However, it should not be forgotten that under- and over-reporting may occur. Under-reporting is not uncommon and it is found that women, elderly and/or overweight/obese individuals tend to under-report their dietary intake [289-293]. There is also evidence for over-reporting in large and small eaters [294, 295]. By adjusting the reported nutrient intake for the total energy intake, these types of reporting errors can be minimized in order to approach more reliable reported data [296, 297]. Recall bias and reduced memory should also be considered.

The FFQs used in EIRA, part II, SLEVIC and SMC have been validated previously and is described in the section "Materials and Methods" of this thesis. The reproducibility as well as the reliability of the FFQs have been taken into account.

6.1.1.2 Missing data

It is not uncommon that participants misinterpret questions or leave response field(s) blank when completing larger questionnaires. There are ways to tackle missing data depending on the power of the study, data content and common sense.

Blank or uncompleted response fields in the FFQ of EIRA, part II and SLEVIC were coded as "missing data". Missing data included also responses with a tick (✓) instead of a frequency number of a food item. The dietary data may have differed if the blank fields or responses with a tick were recoded into "zero intake". However, similar results as in **Paper I** were shown from a sensitivity analysis where a subgroup of only participants with more than 60% (~3rd tertile) of missing data were included (data not shown). Further, the proportion of missing data below and above 60% were compared across subgroups of age, gender, area of residence, BMI, education, EULAR response etc. Missing data of more than 60% were more common in elderly (70+ years) and in men, nevertheless, all the analyses based on data from EIRA, part II were adjusted for age and gender.

In **Paper III**, missing data and/or partial non-response for food items in the FFQ of SMC were recoded as zero intake. Incomplete answers when reporting food intake have been found to be fairly related to null consumption if the food items of the FFQ is not meant to be representative of a diet of the total study sample. [286].

6.1.2 Statistical analysis

6.1.2.1 Logistic regression

Binary logistic regression was used in **Paper I**, **Paper II**, **Paper IV**, and **Paper V** in order to test the association between dietary nutrient intake and clinical outcome. This analysis enabled the measurement of the strength of an association between independent variables(s) and a dependent variable and is often used in case-control studies and when analyzing cross-sectional data. Logistic regression can use fairly small numbers of cases/observations in the model and considers confounding factors. It also uses only valid data in the analysis, however, all the missing data of the dependent variables were excluded before analysis procedure.

6.1.2.2 Linear mixed models

Linear mixed models were used in **Paper III** in order to investigate potential dietary changes after RA diagnosis. Mixed models focused on repeated measures over time of dietary intake in 1997 and 2009. The major advantages of mixed models, compared to student's t-test and/or analysis of variance (ANOVA), are the management of missing data [298], and the ability to adjust the analysis for potential confounders [299]. Mixed models enabled to compare the dietary intake between the two time points, despite the large difference in sample size of women with and without RA.

6.1.2.3 Hierarchical cluster analysis

In addition to mixed models, hierarchical cluster analysis was used in **Paper III** in order to compare patterns of dietary changes between women with RA and matched controls. This type of cluster analysis is used to identify similar characteristics of interest (in this study: dietary changes from 1997 to 2009) within the total sample size [300], and has been widely used in order to detect dietary patterns in larger populations [301].

6.1.2.4 Confounding

Confounders are factors that are related to both the outcome (i.e. disease) and the exposure (i.e. nutrient intake). They can be known risk factors of the outcome and known covariates of the exposure. Several confounders were used for analysis adjustments in **Papers I-V**. The confounders were chosen based on earlier evidence of their link to the outcomes and the exposures used in the analyses. In addition, these were regarded as potential confounders since they were, one by one, significantly associated with the outcome of interest in a univariate analysis. Confounder(s) that would correlate with other confounders in the same model were

excluded in order to avoid over adjustments that could lead to either false positive or negative associations.

6.1.3 Strengths and limitations

6.1.3.1 Paper I: Vitamin D, omega-3 FA, folate and treatment results

This study was the first to investigate the dietary intake of vitamin D, omega-3 FA and folate prior to DMARD initiation, and their associations with treatment results in patients with early RA. The benefits of Vitamin D and omega-3 FA in RA have been frequently studied due to their anti-inflammatory properties, but rarely in regards to anti-rheumatic treatment. This study included a large number of participants that were representative of RA patients in Sweden and the estimated nutrient intake of the FFQ has been validated.

The estimated intake of the three nutrients was assessed close to the start of treatment. This may have introduced non-differential misclassification of exposure, which would result in the ORs for the comparisons between the extreme groups (4^{th} versus 1^{st} quartile) being biased towards the null value. However, dietary patterns were assumed to be unchanged during the first three months from baseline. Clinical RA manifestations, treatment history, doses, adherence and side effects of DMARDs from treatment initiation was not considered in this study. The majority of the patients were treated with MTX, the number of patients with other DMARDs were limited in order to perform sensitivity analysis in subgroups of other DMARDs.

6.1.3.2 Paper II: Diet and GC treatment in SLE

This study is the first to highlight several associations between specific dietary nutrients and GC treatment that might be of clinic importance in patients with SLE. The results presented particular dietary nutrients that were inversely associated with unfavorable outcomes of GC.

The duration of the disease and treatment use varied among patients. Since this study included patients with established SLE, it was assumed that the dietary patterns were unchanged throughout the 2-year study period. GC treatment were used as a proxy of the disease activity, but it may not have fully reflected the activity during the study period. Clinical manifestations, treatment history and side effects of GC throughout the disease course from diagnosis were not considered in this study. This study was unable to examine the association between dietary nutrients and other anti-rheumatic treatment due to the small subgroups of patients with different DMARDs.

6.1.3.3 Paper III: Dietary changes after RA diagnosis

This study is the first to assess the long-term changes in diet after RA diagnosis from a large population-based cohort. The SMC has a strong prospective design including a large population as well as highly validated FFQs. The participants of the SMC were representative of elderly women from the general population in 2009, however, the younger generation may have been more prone to change their diet after RA diagnosis, as hunger, appetite and food intake are affected by age [302].

Long-term changes in diet were based on dietary assessment from only two time points (1997 and 2009), with twelve years apart. Therefore, any important short-term changes during this period were not registered. Women who had been diagnosed with RA before 1997 (prevalent cases) were not excluded from this study since the identification of *all* these women were very limited. The prevalent cases could be tracked by the Outpatient Register, the SRQ and the Inpatient Register. However, the Outpatient Register was initiated only in 2001 and the SRQ was initiated in 1996. The Inpatient Register only provided a small number of prevalent cases since RA is not a disease that usually leads to hospitalization. Nevertheless, it was possible to identify 156 prevalent cases. However, the aim of this study was to evaluate changes in diet due to the diagnosis of RA, in this case it was believed that excluding prevalent cases was not essential since any important changes in diet among prevalent cases may have already been made before 1997. Though, results from a sensitivity analysis where the prevalent cases were excluded did not change the main results (data not showed). Clinical aspects of RA were not considered due to large proportion of missing data (~70%).

6.1.3.4 Paper IV: PUFA and pain in RA

This study was the first to examine the association between dietary intake of omega 3-FA, omega-6 FA and Refractory Pain in a large group of early RA patients. Subjective pain, such as Refractory Pain, was taken into account since it is one of the major problem of the disease from a patient's perspective, and this study found that this particular type of pain may be reduced by omega-3 FA. The estimated nutrient intake of the FFQ have been previously validated.

Clinical manifestations, treatment history, adherence, doses and side effects of MTX from diagnosis were not considered in this study. Moreover, there were no data on depression or psychological factors that are known to be associated with self-reported pain in RA [303]. However, due to the registry-based approach, it is believed that the impact of depression on the

current results may be limited. VAS indicated overall pain and not disease related pain. In this study, Refractory Pain was defined as non-inflammatory pain; however, CRP below 10 mg/L may still indicate some active inflammation.

6.1.3.5 Paper V: Micronutrients and atherosclerosis in SLE

This study is the first to explore whether dietary micronutrients were linked to SLE activity, and the first to provide data suggesting that specific micronutrients may be linked to atherosclerosis in patients with SLE.

The formation of carotid plaque can take several years. Patients included in this study had various disease durations and therefore it was not possible to assume that the reported dietary habits from the FFQ were fully associated with plaque formation. However, this study assumed that the dietary patterns in the study participants were the same at least during the year prior to study inclusion. Some micronutrient intakes were below the RDI. However, dietary supplementation was not taken into account, and nutrient intake from supplements may have covered generously the RDI. Specific clinical manifestations and treatment history were not considered in this study.

6.2 MAIN RESULTS AND INTERPRETATIONS

6.2.1 Paper I: Vitamin D, omega-3 FA, folate and treatment results in RA

The main results showed that higher intake of dietary vitamin D and omega-3 FA during the previous year from DMARD initiation may associate with better treatment results in early RA patients. Higher dietary folate intake was not associated with worse response to MTX in particular.

6.2.1.1 Vitamin D

Higher dietary intake of vitamin D was associated with good EULAR response. A similar study, performed in Philadelphia, USA, showed no association between vitamin D concentration levels and clinical response to therapy using the ACR response criteria in treatment naïve RA patients [304]. Only 15-20% of the vitamin D in blood is related to diet, the rest is produced during sunlight exposure. Vitamin D deficiency measured in blood is common in patients with RA and has been associated with disease activity and inflammatory markers [116, 117]. Vitamin D deficiency in RA patients in Sweden might be an issue due to less sun exposure, as there is only enough UV radiation from the sun to produce vitamin D during only 6 months per year. Therefore, increased vitamin D intake through either diet or supplementation might be of importance. This study suggests that increased dietary intake of vitamin D before and/or during DMARD start may be associated with improved treatment outcome in RA patients. This finding requires confirmation.

6.2.1.2 Omega-3 FA

Dietary omega-3 FA intake was associated with good EULAR response. Evidence suggests that long-chain omega-3 FA have anti-inflammatory properties and are beneficial in the treatment of autoimmune and inflammatory conditions [228, 229]. Combination of MTX and omega-3 has shown a significant reduction in liver enzyme activities [230]. A recent study has also suggested that biomarkers of omega-3 FA may predict clinical outcomes related to standard treatment of RA patients [305]. In addition to the anti-inflammatory effect of the DMARDs, omega-3 FA may have a supplementary role in achieving better treatment outcome in early RA.

6.2.1.3 Folate

The majority of the patients were treated with MTX, which is an anti-folate agent. One of the hypothesis was that the efficacy of MTX may be inhibited by higher dietary intake of folate.

In contrast, this study showed that higher folate intake did not associate with worse treatment response, in particular to MTX. Folic supplementation along with MTX is necessary and has shown to prevent side effects of the drug in RA [73, 74]. Folate fortification in food items such as flour, rice, pasta and other grain products has been common in the last two decades in order to primarily prevent neural tube defect in unborn children, but not in Sweden [306, 307]. However, folic acid fortification in foods has been associated with requirement for higher MTX dose in a small study of RA patients [308].

6.2.2 Paper II: Diet and GC treatment in SLE

Dietary intake of vitamin D did not associate with reduced SLE activity, but alcohol intake did. Beta-carotene (antioxidant), linoleic acid (omega-6 FA) and vitamin B_6 were inversely associated with increased GC dose. The association between dietary intake and higher GC dose levels indicated GC's influence on increasing appetite.

6.2.2.1 Vitamin D, alcohol - GC treatment

Previous studies have shown that low vitamin D levels in serum are associated with higher disease activity in patients with SLE [309-313]. Vitamin D supplementation has been suggested to lower the disease activity and fatigue scores in SLE [314, 315]. Based on these and other studies, vitamin D may be protective against SLE flares. However, in this study, dietary vitamin D was positively associated with GC treatment, not suggesting a protective effect, but this association was not significant after adjusting for supplement use of calcium/vitamin D supplementation. A reason for this results could be that patients with established SLE may have been aware of the existing evidence of the beneficial effect of vitamin D in inflammatory diseases, explaining an increased vitamin D intake in patients with GC treatment/higher disease activity. Nevertheless, these results were based on dietary intake and not supplement intake of vitamin D.

In contrast, alcohol was inversely associated with GC treatment reflecting older findings on the link between moderate alcohol intake and reduced risk of SLE [219, 221, 316]. However, even though alcohol in moderation has shown to be advantageous in some cases, it remains a delicate topic and recommendation of alcohol intake for beneficial effect should be expressed with caution.

6.2.2.2 Linoleic acid, beta-carotene, vitamin B_6 - unchanged/increased GC dose

Linoleic acid was inversely associated with unchanged/increased GC dose (unfavorable outcome). Linoleic acid belongs to the omega-6 FA family and is known to have pro-inflammatory properties. However, omega-3 FA, which is anti-inflammatory, should be considered when looking at the omega-6 FA intake since the ratio between these two must be well-balanced. The omega-6 to omega-3 FA ratio did not show any association with unchanged/increased GC dose. Though, there is gathered evidence on health benefits of conjugated linoleic acids that may be inversely associated with unchanged/increased GC dose [317].

An inverse association was found between beta-carotene and unchanged/increased GC dose. Beta-carotene is an anti-oxidant and SLE patients have been found to have lower dietary beta-carotene intake compared to controls [318]. Increased antioxidant intake could be beneficial due to the damage of free oxygen radicals that play a role in SLE [319, 320]. Beta-carotene may be partially linked to protective effect against unchanged/increased GC dose.

Vitamin B_6 was inversely associated with unchanged/increased GC dose. Higher intake of vitamin B_6 and dietary fiber may prevent the occurrence of active disease in SLE patients [208]. Pyridoxal phosphate (PLP) is the active co-enzyme of vitamin B_6 and its concentration in plasma is the most common measure of vitamin B_6 status [321, 322]. Decreased plasma PLP levels have shown to be associated with chronic or acute disease and increased plasma PLP levels with lower CRP [323-325]. Higher intake of dietary vitamin B_6 may decrease the CRP levels and therefore decrease the SLE activity.

6.2.2.3 Dietary intake - GC dose levels

Energy intake was associated with higher GC dose levels, explaining the association between almost all the nutrients and higher GC dose levels. Contradictory results exist on the effects of GC therapy on energy intake, appetite, and body weight in humans [326]. GC has shown to be associated with increased appetite and/or body weight [260]. Increased appetite during or after GC treatment have been self-reported as one of the major adverse events in several studies [261-263]. Results from this study confirm the existing evidence of GC's influence on increasing appetite.

6.2.3 Paper III: Dietary changes after RA diagnosis

The main results showed that women who had been diagnosed with RA had similar dietary patterns over time as the general population, and these women did not remarkably change their diet due to their disease.

6.2.3.1 Dietary changes

The majority of the results did not show significant changes in diet between 1997 and 2009. The very few significant results from linear mixed models showed that women without RA increased their intake of whole wheat bread, rice and wheat/oat bran more than RA patients. Based on these findings, it was speculated that some of the women with RA may have been treated with GC and therefore had increased appetite, which may have increased their intake of refined graines rather than whole grains. However, further analysis did not show a difference between refined and whole grains between women with and without RA.

6.2.3.2 Dietary interventions in RA

Earlier dietary interventions in RA patients have showed varied results. Fasting during 7 to 10 days before commencing with lactovegetarian and vegan diet, respectively, may decrease both objective and subjective symptoms [327, 328]. Another study has performed dietary manipulation by initially allowing only foods that RA patients are unlikely to be intolerant to, followed by adding other foods one by one, and the results showed also objective and subjective improvements. However, patients were followed for only six weeks [329].

Long-term dietary changes in RA patients and the effect on the disease activity has been examined by Kjeldson-Kragh J et al. The patients were initially introduced 7 to 10 day of fasting followed by gluten-free vegan diet and then lactovegetarian diet over thirteen months. After completing the trial, the dietary changes improved clinical measures compared to unchanged diet [330]. An additional follow-up study was performed on the same participants after two years. Those patients who had adopted and continued with the new diet after two years had still a major reduction of clinical measures, compared to controls with unchanged diet [331].

Many recent studies have focused on PUFAs and highlighting the importance of the composition of the dietary fat intake in RA. As mentioned earlier, omega-3 FA from either diet or supplements have been associated with decreased disease activity in RA, through anti-inflammatory mechanisms [125-128, 130]. Therefore, some interventions have focused on

changing the composition of dietary fat intake by increasing omega-3 FA intake [129, 332, 333], decreasing omega-6 fatty acid intake [334], decreasing saturated fat intake [335], and/or encouraging the Mediterranean diet [104, 336].

Several interventions have reported improvement of the disease status in RA to some extent, however, the power and the duration of the interventions need to be considered. Small number of study participants and short-lasting interventions may not always be representative in an epidemiological context. Based on earlier evidence on the advantages of an improved nutritious diet in RA, specific dietary recommendations for patients with RA are needed.

6.2.4 Paper IV: PUFA and pain in RA

The main results showed that higher intake of omega 3 FA was inversely associated with Refractory Pain and that higher omega 6 FA to 3 FA ratio was directly associated with this Refractory Pain. Omega 6 FA alone did not significantly associate with Refractory Pain. Neither omega 3 FA, omega 6 FA nor omega 6 FA to 3 FA ratio were associated with key inflammatory parameters at three months.

6.2.4.1 Pain in RA

Pain is a major burden of RA and often brings the patient to the healthcare for the first time. In earlier literature, pain does not always correlate with the course of inflammation in RA. For instance, DAS28 remission does not exclude the persistence of significant pain [45], and remaining pain has found to be common in early RA despite anti-rheumatic treatment [337].

High frequency of pain after anti-rheumatic treatment is also supported by other reports. For example, chronic widespread pain has shown in over a third of patients with established RA [338], and has been associated with high levels of pain, fatigue and sleep problems [339, 340], especially during the first year after RA diagnosis [341, 342]. In addition, higher prevalence of fibromyalgia is more common in patients with RA than the general population [343, 344].

6.2.4.2 Refractory Pain

This study focused on pain in spite of inflammatory control after three months of MTX treatment and was defined as Refractory Pain. Although pain reduction is expected after MTX initiation in a majority of early RA patients, the data showed that 15.6% of the patients still experienced Refractory Pain after three months.

Refractory Pain was based on the well-defined PASS, which was earlier described and

validated as the level of definite unacceptable pain. However, PASS alone is a subjective measure and therefore an additional objective measure was considered that could differentiate non-inflammatory pain from inflammatory pain. Low inflammation was defined as CRP level below 10 mg/L. According to the ACR/EULAR definition of remission in RA, a CRP of 10 mg/L or less has been associated with low inflammatory RA core set measures, such as ESR, SJC and DAS28, within the remission interval [281].

Patients with Refractory Pain had higher HAQ score, patients' global assessment score and TJC at baseline and after three months compared to patients without Refractory Pain and these results are in line with previous findings [337, 345]. HAQ scores have been observed to be higher in RA patients with fibromyalgia [346]. Patients' global assessment and TJC have shown to strongly correlate with pain and DAS28 in RA patients [347, 348].

6.2.4.3 Omega-3 FA - Refractory Pain

Omega-3 FA intake was associated with significantly decreased risk of Refractory Pain. Omega-3 FA is known to be anti-inflammatory [125-128], but has not been linked before to non-inflammatory pain in RA. Omega-3 FA may also have an inflammation-independent actions on pain, supported by the lack of associations between omega-3 FA and inflammatory parameters at the three months follow-up. In addition, omega-3 FA has earlier been linked to the production of mediators involved in pain suppression. Resolvins, protectins and lipoxins are omega-3 FA derived non-classical eicosanoids with anti-inflammatory properties. Interestingly, resolvins (e.g. RvE1, RvD1) have shown to directly suppress pain in experimental models [131, 132]. These effects are mediated through inhibition of the actions of transient receptor potential (TRP) cation channels, such as TRPV1 and TRPA1 (16), both known to be strongly implicated in nociceptive mechanisms [349, 350]. Based on the direct effects of RvD on nociceptive mechanisms, it may be hypothesized that higher levels and activity of these mediators related to an increased intake of omega-3 FA may result in direct effects on pain perception, although the exact clinical mechanisms need to be further explored.

6.2.5 Paper V: Micronutrients and atherosclerosis in SLE

The main results did not find differences in dietary micronutrient intake between SLE patients and controls, and between patients with lower and higher disease activity. Dietary intake of riboflavin, phosphorus, selenium and thiamin were inversely associated with atherosclerotic/echolucent plaque. In addition, patients without any atherosclerotic plaque on

the left side had significantly higher intake of several micronutrients compared to patients with atherosclerotic plaque on the left side. These associations were not found in controls.

6.2.5.1 Left sided carotid plaque

A novel observation was that atherosclerotic plaques were more common on the left side in SLE patients than controls. It has been previously reported that IMT in the left side of common carotid artery is increased compared to the right side [351]. One possible explanation of thicker IMT on the left side may be because of a more rapid development of atherosclerosis on this side due to the difference in gross anatomy of the left and the right side. Also, the left common carotid artery originates directly from the aortic arch, which may create other shear stress conditions on the left compared to the right side. Shear stress has been shown to be related to IMT [352].

6.2.5.2 Riboflavin

Lower intake of riboflavin (vitamin B_2) was associated with atherosclerotic plaque on the left side. One of the first studies on the association between riboflavin and atherosclerosis was published in 1968 by Borets VM et al. [353]. The authors studied the influence of riboflavin in three groups and found that a) riboflavin does not influence the coagulation in atherosclerotic patients, b) riboflavin accelerate the coagulation in patients with atherosclerosis and hypertension, and c) simultaneous intake of riboflavin and anticoagulants is beneficial for patients with atherosclerosis and hypertension

6.2.5.3 Phosphorus

Lower intake of phosphorus was associated with atherosclerotic plaque on the left side. Contradictory results have been published regarding the impact of dietary phosphorus on blood pressure and CVD. Some studies claim that dietary phosphorus intake affect serum levels of phosphorus [354, 355], and some do not [356, 357]. Excessive intake of dietary phosphorus from mostly processed food as well as elevated serum levels of phosphorus have been associated with increased risk of CVD [358-360]. However, higher intake of phosphorus has been associated with lower blood pressure and decreased risk of hypertension [361, 362].

6.2.5.4 Selenium

Higher intake of selenium was inversely associated with atherosclerotic plaque on the left side. Selenium has anti-oxidant properties [363]. Serum levels of selenium in SLE patients have

been reported to be lower compared to healthy controls [206, 364]. High-selenium lentil diet has shown to protect against arsenic-induced atherosclerosis in a mouse model [365]. Increased selenium through diet or supplementation may be beneficial for SLE patients.

6.2.5.5 Thiamin

Higher intake of thiamin (vitamin B_1) was inversely associated with atherosclerotic and echolucent plaque on the left side, respectively. Increased dietary intake or supplementation of thiamin may delay the atherosclerotic complications of type 2 diabetes [366]. Thiamin levels in SLE patients have not been well studied. However, SLE and type 2 diabetes are both associated with increased risk of CVD than the general population. Therefore, thiamin may be beneficial for the SLE patients. Echolucent plaque has been associated with vulnerability [367]. The finding on association between thiamin and echolucent plaque is of importance since thiamin may associate with echolucent plaque formation in SLE patients.

6.2.5.6 Folate

Surprisingly, folate was associated with bilateral echolucent plaque, although several studies suggest that folate intake (including folic acid supplementation and fortification) has an important role in improving cardiovascular health [368]. Folate deficiency has been associated with increased carotid IMT, which can be used as a marker of atherosclerosis and vascular disease [369]. However, folic acid supplementation has not shown any influence on the coagulation, inflammatory and lipid parameters in subjects with atherosclerosis risk factors [370]. The association between dietary folate and carotid plaque, especially in SLE, remains unclear.

The associations discussed above were typically found in SLE patients but not in controls, this indicates that these particular micronutrients may be specifically important in SLE.

6.3 ETHICAL ASPECTS

6.3.1 Confidentiality

Papers I-V have been approved by the Regional Ethical Review Board at Karolinska Institutet, Stockholm, Sweden. All dietary data used in **Papers I-V** were based on previously collected data from EIRA, SLEVIC, and SMC. All the participants of these registers had been asked to complete a consent form before inclusion, covering additional approval to share their data for further studies. The personal identification numbers of the participants from EIRA and SMC had been re-coded and were not available for external investigators who wish to perform further research based on the collected data. However, personal identification numbers of the patients from SLEVIC were available since additional clinical data were completed from medical records in **Paper II**. The data extraction from medical records may have been delicate since additional and/or unrelated information about the patients' medical profile could not always have been avoided. Nevertheless, the data extraction was performed with confidentiality and was taken with respect.

6.3.2 Dietary assessment

FFQ used in EIRA, part II, SLEVIC, and SMC were voluntary to complete. No ethical issues of the FFQ were considered since the participants were not being asked to follow a specific diet, they just reported their food intake. Some patients may have felt uncomfortable about reporting their dietary habit since it might be a sensitive topic. However, the FFQ was voluntary and the reported data were not shared with the patients' caregiver(s).

7 CONCLUSIONS

Results from this thesis presented several associations between specific dietary nutrients and clinical outcomes of RA and SLE, in particular concerning treatment results. In summary, diet may play a role in response to anti-rheumatic treatment in patients with RA and SLE. Conclusions of **Papers I-V** are summarized in table 26.

Table 26. Conclusions of **Papers I-V**.

Paper	Description	Conclusions
I	Vitamin D, omega-3 FA, folate and treatment results in RA	• Increased intake of dietary vitamin D and omega-3 FA may associate to better treatment results of DMARDs in early RA patients. • Dietary folate intake was not associated with worse response to treatment, in particular of MTX.
II	Diet and GC treatment in SLE	• Dietary vitamin D did not associate with decreased lupus activity. • Beta-carotene (anti-oxidant), linoleic acid (omega-6) and vitamin B_6 may protect against unfavorable outcomes of GC (increases in dose). • The association between dietary intake and higher GC dose levels indicated GC's influence on increasing appetite in SLE patients.
III	Dietary changes after RA diagnosis	• Women who had been diagnosed with RA had similar dietary patterns over time as the general population, and did not specifically change their diet due to their disease. • Based on earlier evidence on the advantages of improved nutritious diet in RA, specific dietary recommendations for patients with RA are needed.
IV	PUFA and pain in RA	• Dietary intake of omega-3 FA may associate with reduced non-inflammatory pain in MTX treated patients with early RA.
V	Micronutrients and atherosclerosis in SLE	• Dietary micronutrient intake did not differ between SLE patients and healthy controls, and between SLE patients with lower and higher disease activity. • Riboflavin (vitamin B_2), phosphorus, selenium and thiamin were inversely associated with atherosclerotic/echolucent plaque in SLE patients. • Certain dietary micronutrients may play a role in atherosclerosis in SLE.

8 FUTURE RESEARCH

This thesis reported several associations between dietary factors and clinical outcomes in patients with RA and SLE. Yet, there are many aspects that remains unclear and need to be explored more. Future research suggestions based on results of **Papers I-V** are listed below.

8.1 PAPER I: VITAMIN D, OMEGA-3 FA, FOLATE AND TREATMENT RESULTS IN RA

- Dietary aspects of anti-rheumatic treatment need to be confirmed in larger scales and even across different populations. For instance, the association between dietary vitamin D, omega-3 FA and treatment outcome of MTX may be studied in different countries and be compiled into a meta-analysis.
- Dietary nutrient intake from food or supplements does not necessarily correlate with the nutrient status (nutrient levels in blood). The correlation between estimated nutrient intake from FFQ and nutrient status should be examined in MTX treated early RA patients.
- This thesis has reported estimated nutrient-drug interactions through FFQ and treatment outcome assessments. However, nutrient-drug interactions need to be tested in vitro in order to fully understand if some specific nutrients inhibit or enhance the efficacy of the drug.
- The study design of **Paper I** should be implemented in patients with early RA treated with biological drugs.

8.2 PAPER II: DIET AND GC TREATMENT IN SLE

- It is not uncommon that patients with RA and SLE experience gastro-intestinal adverse events due to either their disease and/or side effects of drugs. Bioavailability and absorption of nutrients in rheumatic patients need to be analyzed and compared with healthy controls.
- The association between diet and treatment outcome (i.e. using SLEDAI or SLAM) should be studied in larger groups of SLE patients, higher power would enable the possibility to examine the associations in different subgroups of traditional DMARDs used in SLE.

8.3 PAPER III: DIETARY CHANGES AFTER RA DIAGNOSIS

- Patients with rheumatic diseases who actively show interest for diet should be provided with relevant information. Based on earlier evidence of reviews and interventions on the advantages of improved nutritious diet, specific dietary recommendations for patients with RA should be established.

- Short-term changes in diet were impossible to track in **Paper III**. Any possible dietary changes, both short- and long-term, after rheumatic diagnosis may be studied closer. It is of interest to inverstigate *why* rheumatic patients voluntarily make dietary changes, *how* long the changes last and *if* any benefits of new dietary patterns have seen. This could easily be studied with short and simple questionnaires regarding dietary patterns after rheumatic diagnosis distributed to patients from outpatient clinics during regular visits.

8.4 PAPER IV: PUFA AND PAIN IN RA

- In addition to the results of **Paper IV**, higher sodium intake was associated with Refractory Pain. However, this association needs to be confirmed. Furthermore, salt intake from urine samples should be assessed for additional validation of the estimated sodium intake from the FFQ.
- The study design of **Paper IV** could be implemented in the patients from SLEVIC in order to investigate if the same trend is seen in SLE patients as in RA patients.

8.5 PAPER V: MICRONUTRIENTS AND ATHEROSCLEROSIS IN SLE

- Lipid profile in SLE patients may vary dependent on diet and GC treatment. The link between diet and GC treatment, and its association to lipid profile in participants of SLEVIC should be examined.

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